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SOME RECENT FINDINGS IN THE INHIBITION OF AVIAN REPRODUCTIVITY

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SOME RECENT FINDINGS
IN THE INHIBITION OF AVIAN REPRODUCTIVITY

SIX SEMINAR PAPERS

by

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D. K. Wetherbee, Editor

A seminar conducted in 1961 by the Department of Poultry Science, University of Massachusetts, and the Massachusetts Cooperative Wildlife Research Unit, supported by the University of Massachusetts, the Massachusetts Division of Fisheries and Game, the United States Fish and Wildlife Service, and the Wildlife Management Institute.

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INTRODUCTION

The problem, in poultry science and in wildlife management, of correcting or of inducing avian reproductive sterility is of great importance inasmuch as the ability or inability of a population of birds to reproduce itself is often the limiting factor in the continuance of that population at levels optimal for economic use, scientific manipulation, population control, or even survival of species. Any consideration of fertility must take note of the causes of sterility. The six papers presented in this seminar are focused upon sterility either as reproductive failure deliberately induced, reproductive failure incidentally discovered, or sterility mechanisms postulated. For the most part, these papers are restricted to reviews of recently published findings, and, while not exhaustive in scope, they were prepared with a view toward presenting a preliminary elucidation of the breadth of subject area involved in reproductive failure in birds. The published literature is heavier in some areas than in others; however, the authors agreed to seek some balance between General Physiology, Genetics, Nutrition, Pharmacology, and Endocrinology. As the subject of avian sterility has hardly been opened for exploitation, the authors felt that some speculation might be virtuous; however, the temptation to extrapolate mammalian research was restricted to those approaches that might be most promisingly applied to avian species.

It is appropriate, we believe, that an initial attempt to summarize literature on avian sterility should be made from this station, the College of Agriculture, University of Massachusetts, for the College is pioneering in avian sterility research both in the poultry science area and in the wildlife management area. Among the faculty and graduate student research projects underway here are the following:

1. Inheritance of fertility and hatchability in turkeys, by F. R. Smyth.

2. Effects of certain organic and inorganic diluents on fertilizing ability of turkey semen, by F. R. Smyth.

3. The effect of mineral balance on requirements of laying chickens for maintenance of egg production and reproduction, by D. L. Anderson.

4. Genetics and physiology of the length of the incubation period in the chicken, by F. R. Smyth.

5. Effect of sperm survival time in the female reproductive tract on fertility and hatchability in chickens, by F. R. Smyth.

6. Sterility in birds produced by immune responses, by B. C. Wentworth.

7. Relationship between low fertility and rose comb in the domestic fowl, by R. D. Crawford.

8. Screening tests of chemical materials having candidacy in producing sterility in birds, by D. K. Wetherbee and M. F. Landy.

9. Hormonal stunting of development of the avian infantile gonadotrophic system, by D. K. Wetherbee.

10. Ecology and physiology of reproduction inhibition in breeding wild bird species, by D. K. Wetherbee and R. P. Coppinger.

11. Radio mimetic sterility in certain avian species, by D. K. Wetherbee.

While the need for continuing research in sterility has long been recognized in poultry science, only recently has wildlife management seized upon the potential rewards of investigation in sterility and fertility. To raise the threshold of productivity of wildlife species to fill improved carrying capacities of managed habitat is an obvious advantage to increasing fertility. A less obvious, but perhaps even more useful, tool in wildlife management is the manipulation of reproductive failure in the control of certain wildlife species. Where reduction of nuisance species is concerned, it has been postulated that the presence of a given number of sterile individuals in a population exerts a much greater biological control pressure on that population than removal of that same number of fertile individuals.

It is the hope of the participants in this seminar that the collection of reviews presented here will serve to stimulate increased investigation in avian sterility and fertility.

RECENT FINDINGS IN THE INHIBITION OF AVIAN SPERM SUSTENTATION

Roy D. Crawford

1. In the male reproductive tract.

   a) Normal sequence of events.
   b) Effect of frequency of collection on semen volume and fertilizing capacity.
   c) Effect of external environment on semen production.
   d) Effect of the "scrotum".
   e) Effect of chemicals.

2. In vitro.

   a) Effect of length of storage and storage temperature on undiluted semen.
   b) Effect of diluents on fresh and stored semen.
   c) Effect of bacterial contamination.
   d) Effect of chemicals.
   e) Effect of copulatory secretions.

3. During copulation.

   a) "Chastity belt" devices.
   b) The copulatory organ.
   c) Effect of aberrant behavior.

4. In the female reproductive tract.

   a) Normal sequence of events
   b) Effect of adverse oviducal environment.
   c) Control of sex.

5. Literature cited.

6. Additional references which may be pertinent.

RECENT FINDINGS IN THE INHIBITION OF AVIAN SPERM SUSTENTATION

Roy D. Crawford

1. In the male reproductive tract.

a) Normal sequence of events.

The functional maturation of spermatozoa has recently been reviewed by Romanoff (1960). The fertilizing capacity of the sperm is gradually acquired as they pass through the male reproductive tract. According to Munro in 1938, as quoted by Romanoff, sperm cells taken directly from the testes yielded 16.1 per cent fertility on insemination, those taken from the epididymis gave 18.8 per cent fertility, and those from the vas deferns were 65.3 per cent fertile. Therefore, like mammalian sperm, avian spermatozoa appear to undergo a period of maturation, this maturation being attained mainly in the vas deferens. Since the cells pass through the reproductive tract at a rapid rate, aging does not seem to be a major factor in the ripening process. According to Romanoff, the nature of the maturation process is not clear -- perhaps protoplasmic changes occur in the spermatozoa, or a protective colloidal coating may be added to the cells as they pass through the vasa. Apparently, the male environment is necessary for the development of fertilizing capacity since immature sperm do not ripen in the oviduct.

Also, as in mammals, avian sperm cells do not show motility within the testes. Motility is acquired during passage through the reproductive tract. The time required for passage varies from one to four days in the chicken, depending upon mating activity. According to Munro in 1938, as quoted by Romanoff, chicken spermatozoa may live and show motility for as long as 30 days if forced to remain in the vas deferens. They will also remain viable in ligated vasa if the testes are removed, indicating that hormones from the testis play little or no part in promoting their survival.

b) Effect of frequency of collection on semen volume and fertilizing capacity.

Since it has been demonstrated that avian sperm undergo a process of maturation before attaining full fertilizing capacity, it might be suspected that frequency of mating or collection of semen may have an adverse effect on fertility. It might be expected that with frequent ejaculation a high proportion of immature sperm would be released.

The relation between frequency of collection and amount of semen obtained from turkey males has been studied by Lorenz, Wilson and Asmundson (1955, 1956). In their first study, they used ten sets of four brothers of the Broad Breasted

Bronze variety. One male of each set was assigned to each of the following treatments: semen collected once weekly, twice weekly, three times weekly, and on various series of consecutive days. They found that volume per sample was not affected except when semen was collected daily. In this case, volume collected per day decreased steadily and then became stable by the fifth consecutive day of collection. Apparently, sperm concentration was not affected by frequency of ejaculation. In the second study, semen was collected at longer intervals -- once each week, once every two weeks, and once every four weeks. They found no difference between treatments in sperm concentration, but found that semen volume was reduced in males ejaculated every two and every four weeks.

These studies were carried a step further by McCartney, Chamberlin, Carter and Wyne (1958), who studied the effects of frequency of semen collection on fertility. They used Small-type White turkeys, the males being ejaculated daily, five days per week. Pooled semen samples were inseminated to a different group of females each day so that the qualities of each day's sample could be evaluated. They found no effect of frequency of semen collection on sperm concentration, but found that semen volume decreased gradually to the fifth consecutive day of collection. Fertility was not affected.

c) Effect of external environment on semen production.

Recently there have been several papers dealing with the effects of season on avian semen. In general, these reports indicate a rather marked influence of time of year on quantity and quality of semen, and might conceivably have some bearing on studies of the inhibition of fertility.

Schindler, Volcani and Weinstein (1957) observed the motility of semen from White Leghorn chickens obtained over a twelve-month period. Semen was collected on three consecutive days of each month and pooled for study. They found that motility of sperm was highest in May; it decreased to October, and then increased to a high level by January. Concurrently, it was observed that molting started in July, was intense in September and ended by November. They also noticed that sperm motility was high in increasing daylight and was reduced in periods of decreasing daylight. They concluded that their data indicated a possible relationship between stage of molt and motility of sperm, as well as a possible relationship to changes in temperature and length of day.

Seasonal variations in semen characteristics of adult Fayoumi chickens were studied by Kamar and Badreldin (1959). They used 18 males, collecting semen twice each month over the course of a year. Measurements were taken of semen volume, motility, concentration, per cent live sperm and per cent abnormal sperm. They found monthly and seasonal variations in each characteristic studied. The best semen quality, as measured

by sperm motility, viability and morphology, was obtained in January and February; the poorest quality occurred during the summer, May to August. The best semen quantity, as measured by semen volume, sperm concentration and number of sperm produced, occurred during the summer, and lowest quantity was obtained during the winter. They expressed the idea that biological factors were mainly responsible for semen production, whereas technological factors were mainly involved in semen quality. Thus, their results could be explained on this basis -- poor semen quality was obtained during the Egyptian summer when extreme weather conditions probably had very adverse effects on the semen samples obtained.

Somewhat similar studies have been conducted by Carson, Lorenz and Asmundson (1955) in studying Broad Breasted Bronze turkeys. They found semen production to fluctuate seasonally, maximum production occurring from February to early summer. During the late summer and fall, most males ceased producing sperm although some continued to produce semen at a low level.

Kosin and co-workers have attempted to separate out one of the components of "season" and have published a series of papers dealing with the effects of temperature per se on reproductive ability of Broad Breasted Bronze turkey males.

In their first study (Kosin, Mitchell, and Burrows, 1952), males were placed in different environments during the pre-breeding season. One group was held in an outdoor pen, and the other in an insulated house held at 65 degrees F., both being subjected to the same amount of artificial light. In the subsequent twelve-week breeding season, it was found that the "pre-heated" males gave consistently higher fertility than those subjected to natural low-winter temperatures.

In the next phase of the experiments (Kosin, Mitchell, and St. Pierre, 1955), temperature of the males was artificially altered late in the breeding season, that is, between May and July. Males were removed from the outdoor breeding pens at 9 a.m., one group being placed in an insulated house held at 65 degrees F., the other being held in an outdoor pen out of sight of the females. Both groups were returned daily to the breeding yards at 4 p.m. It was found that this daily cooling temporarily halted the seasonal downward trend in fertility. They demonstrated that during the day the males in the insulated house had lower body temperatures than those in the outdoor pen.

Semen quality studies of males held under the two environments, controlled and outdoor temperature, were reported by Law and Kosin (1958). In this work, 80 males were subjected to each environment. Semen was collected every two weeks from a group of males under each treatment for a period of two years. During the first year, two males of each group were sacrificed every two weeks for histological study of the reproductive

tract. The controlled temperature group showed no drop in sperm concentration during the first fall as was found in the males housed outdoors. During the second winter, the outdoor group produced no semen but by the following spring, they exceeded the controlled temperature group in sperm concentration. This indicated to the authors that a "rest period" was essential for normal sperm production in the turkey male. There was less fluctuation in size of the seminiferous tubules in males housed at a constant temperature.

d) Effect of the "scrotum".

The thermo-regulatory function of the scrotum in mammals is well known. Sterility usually results when the testes are retained in the body cavity. Birds do not have a structure homologous to the mammalian scrotum and it has remained somewhat of a mystery why fertility of birds should be high when the testes are held within the body cavity.

It has been postulated that the posterior abdominal air sacs may exert a colling effect on the testes and, in effect, assume the function of a scrotum. This was tested experimentally by Herin, Booth and Johnson (1960) by surgically removing the air sacs, and by removing air sacs and insulating the testes with surgical cotton. They found no differences in semen quality between treated birds and controls. Semen quality was measured on the basis of motility, concentration, morphology and per cent of dead sperm cells. They further found no differences between testicular, air sac and rectal temperatures. They concluded that the air sacs do not cool the testes of the rooster.

In passerine birds, there is another structure which may have a thermo-regulatory function. In these birds, during the breeding season, there is an extensive development of the cloacal region produced largely by growth and coiling of the lower end of the vasa deferentia. The structure closely resembles a scrotum and testes from external appearance. Wolfson (1954 and 1960) has obtained temperature measurements of the body cavity (taken in the large intestine) and of the cloaca in four passerine species. In each species studied, cloacal temperature, in the immediate vicinity of the "scrotum" was seven degrees Fahrenheit cooler than the temperature of the body cavity. Wolfson postulated that in passerines this structure serves as a thermo-regulator for maintenance and maturation of sperm. This hypothesis has not been tested experimentally, but such testing would no doubt be of considerable interest and might throw additional light on this perplexing problem.

e) Effect of chemicals.

Few recent reports have been located in which a drug or chemical was found to affect the sustentation of sperm adverse-

ly in the avian male reproductive tract subsequent to spermatogensis. It does not seem unreasonable to expect that many such substances could be found. They might express themselves by inhibiting maturation of sperm, by reducing or inhibiting the development of motility, or by altering the seminal plasma produced by the seminiferous tubules and the epididymis. The effects of chemicals upon semen in vivo are amenable to various laboratory determinations of morphology, motility, relative numbers of spermatozoa, volume, viscosity, etc. that have been perfected for use in other animals. Kamar (1959) developed a method that differentiates living from dead sperms in fowl and that gives a preliminary picture of the viability and fertility of semen samples within minutes after semen collection.

Jackson et al (1961) have shown that oral administration of methylethanesulphonate and methylmethanesulphonate inhibit fertility of spermatozoa in rats. The mode of action is unknown.

2. In vitro.

a) Effect of length of storage and storage temperature on undiluted semen.

There have been many reports, some of them quite recent, indicating that storage of semen is highly deleterious to its fertilizing capacity. As a result, research has been directed towards finding the maximum time that semen can be held without total damage and towards finding the optimum temperature for such storage.

Garren and Shaffner (1952) studied the effect of temperature and time of storage on the fertilizing capacity of undiluted fowl semen. They used New Hampshire chickens, storing semen at 0, 10, 20, 30, and 40 degrees Centigrade, and inseminating it at 90-minute intervals. In their work, the optimum storage temperature was found to be 10 degrees Centigrade for up to six hours of storage time. When stored at 15 degrees Centigrade, fertility was maintained for three to four hours. Sperm stored at 0 and 5 degrees Centigrade showed the best retention of motility but their fertility dropped sharply after a few minutes of storage. The fertility of fresh semen was consistently better than that of stored semen.

Somewhat similar results were found by Hunsaker, Aitken and Lindblad (1956) using White Leghorns. They found that fertility declined as the length of the holding period increased, the rate of decline depending upon the holding temperature. When stored at 30 degrees Centigrade, there was an immediate and rapid decrease in fertility. At 0 degrees Centigrade, high fertility levels were maintained for two hours but they dropped quickly thereafter.

Working with Beltsville White turkeys, Harper (1955) found that length of holding period was not critical early in the breeding season but that it was later. He had compared the fertility and duration of fertility from pooled semen stored at about 60 degrees Fahrenheit for one, two, and four hours and from fresh semen.

Carter, McCartney, Chamberlin, and Wyne (1957) studied the fertilizing capacity of semen from Large White turkeys held at 10 and 15 degrees Centigrade for 0, 2, 4, and 6 hours. They found little difference between results obtained at the two storage temperatures but found a fairly consistent sharp drop in the fertility of semen held for two hours or longer.

b) Effect of diluents on fresh and stored semen.

Studies under this heading have been aimed almost exclusively at finding diluents which will give satisfactory results in extending the use of fresh semen and diluents which will prove satisfactory for storing semen. The literature on this subject is too voluminous and detailed to be covered adequately here. The paper by Wilcox and Wilson (1961) provides a bibliography from which a review of the literature on this topic might be begun.

c) Effect of bacterial contamination.

Contamination of semen samples with bacteria is a major problem in studies of sperm metabolism. Some research has been directed towards finding methods of circumventing the problem. Wilcox and Shorb (1958) found that the addition of oxytetracycline and dihydrostreptomycin to stored semen diluted with a buffer resulted in improved fertility.

Although they presented no data as such, Gale and Brown (1961) stated that the presence of bacteria in semen and their multiplication probably result in deterioration of semen samples <u>in vitro</u>. They cultured samples of turkey semen and found a great variety of bacteria. Staphylococci were present in 78% of the samples and coliforms in 53% of the samples. They found that seminal fluid taken aseptically from the vas deferens contained no bacteria and concluded that the cloaca was the source of contamination.

d) Effect of chemicals.

Reference has been found to one chemical which might be regarded as having both beneficial and deleterious effects on semen <u>in vitro</u>. Blackwood and Harris (1960) found that 2,5-alkyl benzimidazoles would inhibit almost completely the motility of chicken sperm. The exact metabolic processes which

were interrupted were not determined but the authors indicated that they were probably quite complex. They investigated the effects of three benzimidazoles, designated DMB, EMB, and HMB, on chicken spermatozoa stored for three days at 10 degrees Centigrade in a phosphate buffer. All three substances were found to inhibit motility. HMB interfered with fructolysis and probably as a result, destroyed the fertilizing capacity of the sperm. However, both DMB and EMB permitted fructolysis to continue, and it was found that after removal of the substances, motility was restored and satisfactory fertility could be obtained even after three days' storage. The fertility levels obtained were: DMB - 60%, EMB - 48%, as compared with fresh semen - 86%.

Harris, Wilcox, and Shaffner (1961) extended the use of these chemicals to turkey semen and found that EMB would inhibit motility in the sperm of this species and that some fertility could be obtained after storage for six hours.

e) Effect of copulatory secretions.

During the past few years, a controversy has been raging over the nature of the transparent fluid obtained when semen is collected artificially from male chickens. Lorenz (1959) has reviewed much of the pertinent information. It has been thought that the fluid is mainly lymph, or lymphlike blood transudate, presumably some of the fluid responsible for erection of the phallus. The amount of this fluid obtained varies widely from different males, the proportion being increased by overvigorous milking at the base of the phallus.

Lake, as quoted by Lorenz (1959), considers the transparent fluid to be an artifact of collection technique and not a normal component of semen. However, Nishiyama (1955) disagrees, believing that it is a normal component corresponding to the secretions of the accessory reproductive organs of mammals. Nishiyama and Fujishima (1961) reported that the fluid is derived from the blood, not from the lymph as Lorenz reports, and that it is ejected during natural copulation.

Lorenz (1959) states that the transparent fluid clots readily, and if present in considerable amounts in artificially collected semen may cause the whole sample to clot. Lake has observed its presence to reduce fertilizing capacity significantly in semen standing at room temperature for twenty minutes. Nishiyama (1955) states that small amounts of the fluid increase the motility of sperm but that large amounts of it result in decreased motility. Clumps of sperm form soon after collection, the size of the clumps depending on the amount of transparent fluid present. Some of the clumps may be caused by an adhesive substance believed to be generated by partial coagulation of the transparent fluid.

A large cloacal gland was described by Coil and Wetherbee (1959) in the Quail (Coturnix coturnix) which exudes a copious mucoid secretion presumably of obligate function in copulation and therefore pertinent to fertility.

3. During copulation.

a) "Chastity belt" devices.

Interest has been expressed in the possibility of using "chastity belt" devices to inhibit the transfer of semen from male to female birds. When studies of methods of semen collection for both artificial insemination and metabolism studies in the domestic fowl were being initiated, several such devices were used and have been described in the literature. Kamar (1958) has made reference to these in his extensive bibliography on methods of collecting semen.

In their studies of the transparent fluid of cock semen, Nishiyama and Fujishima (1961) made use of a collecting device to obtain semen samples from natural matings. It consisted of a small vinyl pouch attached to an oval wire ring, five centimetres in diameter. An elastic sling was attached to the sides of the collector and extended around the base of both wings of the bird. The collector covered the vent and the processes of both pubic bones and was held fairly well in place by them. "If the elastic sling was stretched in equal intensities on both sides and was not too loose, the collector scarcely slipped from the vent during the mating action, even when the cock tried vigorously. This semen collector was very light and the semen could be collected in a state of natural copulation."

b) The copulatory organ.

Wolfson (1960) described the ejaculate of passerines as a viscous drop of material which is essentially a "solid" mass of motile sperm. He further described a penile organ that serves in actually intromitting the relatively small amount of ejaculate. The presence of a penis-like organ has long been recognized in waterfowl and the hormonal control of the "penis" has recently been elucidated by Hohn (1960). Coil and Wetherbee (1959) described a penile structure in the Quail (Coturnix coturnix). The existence of an intromissive organ in birds presents another mechanical and/or physiological site vulnerable to manipulation in thwarting fertility.

c) Effect of aberrant behavior.

Aberrancy in birds may have a basis in hormones and faulty behavior releasing mechanisms. Reference has been found to three studies in which aberrant behavior seems to be impli-

cated as a major cause of low fertility. The first of these is a study by Parker (1961) of the low fertility problem in Cornish cockerels. He compared the fertility in flock matings of New Hampshire, Delaware and Cornish cockerels and found that the fertility from Cornish males was 10 to 12 per cent lower than that from males of the other breeds. Similar results were obtained in another year. When the fertility of Cornish males was compared with that of New Hampshire males and with New Hampshire x Delaware males in flock matings, again Cornish fertility was low. However, when matings were conducted using artificial insemination, the fertility of the Cornish was equal to that of other males indicating that the fertilizing capacity of the semen was not at fault. In single male mating pens, it was found that Cornish males failed to mate with about 8 per cent of their flock mates; and in flock-mated pens, the same was found to hold true. It was further found that certain females tended to remain unmated during much of the breeding season when mated to Cornish males but not when mated to New Hampshire males. Parker concluded that part of the low fertility of Cornish males was due to their failure to inseminate naturally a substantial percentage of their flock-mates.

In studying factors which affect fertility in Jersey Buff turkeys, Smyth and Leighton (1953) found that the percentage of total successful matings of males was of major importance in determining fertility. They found a correlation coefficient of 0.50 between the percentage of completed matings and fertility. In their study of seven turkey toms, mating efficiency was found to vary from 44.3 to 87.5 per cent. Efficiency was considered to be a trait of the male, the female having little influence or responsibility in this regard. The males with the highest per cent of completed matings had good balance, were patient and completed matings with a minimum of difficulty. Males with low percentages of completed matings had poor coordination by comparison, and were characterized by peculiar mating habits -- for instance, treading the floor and treading the female when oriented in a head-to-rear position.

Hale (1955) conducted a somewhat similar study using White Holland turkeys. He agreed that efficiency of males in completing matings was very important in the expression of fertility since if the mating were interrupted at the stage where the male treads and the female everts, the female will no longer be sexually receptive and hence will likely be infertile. He found a correlation coefficient of 0.79 between fertility and efficiency of males in inseminating females during attempted matings. The males studied showed marked individual variations in their efficiency. Low social rank contributed to reduced efficiency. Some of the best males were of the Broad Breasted Bronze variety and some of the worst were Beltsville Small Whites indicating that size and conformation are perhaps not of such great importance as originally thought. Males characterized by low sex drive frequently tramped the neck of the female

or treaded the floor near the female's head rather than mounting. Some males behaved normally at first, but later showed aberrant behavior, which might have been an expression of low sex drive.

4. In the female reproductive tract

a) Normal sequence of events.

Lorenz (1959) has reviewed the normal sequence of events in the female reproductive tract. During natural mating, semen is deposited on the everted vagina. During artificial insemination, semen is usually deposited in the vagina, but techniques are available for both intra-utero and intra-peritoneal insemination. It is believed at present that sperm rely on their own motility to pass through the uterovaginal junction, but come under the control of some other transport mechanism to reach the infundibulum. Van Drimmelen, according to Lorenz (1959), was the first to advance the idea that sperm are stored in "sperm nests" in the oviduct, and this has achieved wide acceptance. He suggested that release of sperm from the sperm nests would occur as a result of stretching the oviduct during passage of the yolk.

b) Effect of adverse oviducal environment.

Apparently, Bernier, Taylor, and Gunns (1951) were the first to suggest that some cases of infertility might be due to adverse conditions in the oviduct. They suggested this in reporting their studies of infertile matings in two inbred lines. They stated that it was probable that certain conditions associated with the females of the inbred lines, possibly conditions in their oviducts, did not permit the ascension of spermatozoa to the infundibulum. Support for this hypothesis came from observations on attempted artificial insemination of a number of completely infertile females; these females remained infertile.

Polin, Porter, and Cobb (1961) studied the effects on reproduction in the domestic fowl of a coccidiostat, glycarbylamide (4,5-imidazoledicarboxamide). They found that both fertility and hatchability were severely depressed at high concentrations of the drug in the diet, but that at lower concentrations reproduction was normal. There was a complete recovery of normal fertility and hatchability four days after removal of the drug from the feed. It could be located in the albumen of the egg, the amounts found increasing with increasing amounts of the substance fed. Although they did not determine definitely how the coccidiostat was acting to depress fertility and hatchability, they suggested the possibility that the sperm may be killed by contact with glycarbylamide lining the lumen of the magnum of the female, or that the glycarbylamide

may exert a gametocidal effect on the ova. A third possibility was that eggs might have been fertilized normally but underwent pre-oviposital embryonic death which was not detected.

A final instance where factors in the oviduct might inhibit the fertilizing capacity of sperm is illustrated in recent work of Grigg and Skaller (1958). In a flock of 350 hens, they found three that were repeatedly sterile on artificial insemination. When these were inseminated intraperitoneally, 90% of their eggs were fertile. They considered that the block to normal fertilization could have been due to failure of the active mechanism of the oviduct in transporting sperm, or to an exaggeration of the restrictive function of the utero-vaginal junction. Since this study was completed, the authors have adopted a new insemination technique, depositing semen near the utero-vaginal junction, and have found no hens to be sterile as a result. They consider this as evidence in favor of the idea of super-restriction of the utero-vaginal junction causing sterility, and hence an example of inhibition of fertility attributable to adverse conditions in the oviducal environment.

c) Control of sex.

Positive control of sex has not yet been obtained in either mammals or birds. Perhaps such control would be easier to obtain in mammals since the male is the heterogametic sex, having both X-bearing and Y-bearing sperm; by eliminating one kind of sperm, presumably only offspring of one sex would be produced following insemination; the factor making such control in mammals easier being that the sperm are readily available for manipulation.

The situation in birds is made somewhat more difficult because the female is the heterogametic sex, males having no part in determining gender of offspring. Thus, to achieve control of the sex of progeny, a means must be found to eliminate either X-bearing or Y-bearing (or O-bearing) ova before fertilization.

One possibility that comes to mind is the use of the antigen-antibody phenomenon. Theoretically, it might be possible to immunize males against the Y- or O-chromosome; upon insemination, the X-bearing sperm would react against Y-bearing ova with resultant infertility, but the sperm would unite readily with the X-bearing ova; the end result would be production of males only, but unfortunately the fertility of eggs so treated would be less than 50%. McCartney (1923) was able to inhibit fertility by injecting rooster sperm into laying hens; the period of infertility lasted from 12 to 67 days after injection. Lamoreux (1940) was unable to repeat these results. No doubt, present techniques will require considerable refinement before this theory of artificial control of sex in birds could be tested experimentally.

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RECENT FINDINGS IN THE INHIBITION OF AVIAN OVA SUSTENTATION

Pedro F. Consuesra

1. Structure of the oviduct and its function.

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RECENT FINDINGS IN THE INHIBITION OF AVIAN OVA SUSTENTATION

Pedro F. Consuesra

1. Structure of the oviduct and its function.

The oviduct is a tube having many loops through which the yolk and germ pass for fertilization and the addition of the white and surrounding membranes. The tube extends from the base of the ovary to the cloaca. The oviduct, which is 2 feet long, varies in structure throughout in length in accord with its various functions, the different portions of this duct being specialized for the secretion of the different parts of the egg.

The oviduct consists of the following five major parts: 1--the infundibulum or funnel which picks up the yolk after release from the ovary, 2--the magnum or albumen-secreting portion, 3--the narrow isthmus where the shell membranes are formed, 4--the pouch-like uterus in which additions to the white are made and the shell is deposited. If the shell is white, the egg is finished after shell deposition, except for the cuticle which is formed just prior to expulsion. In breeds that naturally lay colored eggs, the pigments are laid down in the shell gland. 5--the vagina which leads from the uterus to the cloaca from which the egg is expelled.

There is no sharp line of demarcation between most of these regions; the magnum comprises over 50 per cent and the isthmus and uterus about 15 per cent.

Warren and Scott (1935) obtained fairly accurate timing of the passage of the egg through the oviduct. The calculated time schedule was 18 minutes in the infundibulum, close to 3 hours in the magnum, over an hour in the isthmus and between 20 and 21 hours in the uterus and vagina.

The development of the oviduct and its preparation to receive the mature ovum are directly controlled by the hormones of the ovary. (Herrick, 1944). As the time of ovulation approaches the individual gland cells and glandular folds enlarge, the ducts and cells become filled with secretory products. Most of the proteins that contribute to the albumen are formed and accumulated at a fairly regular rate in the tubular glands of the anterior portion of the oviduct during the interval between successive eggs (Conrad and Scott, 1942).

3. Factors affecting the structure of the oviduct.

Finne and Vike (1951) described an hereditary atresia of the oviduct in a flock of young White Leghorn chickens in Norway. The females were apparently of normal development but

they were unable to lay eggs because the oviduct was ruptured at the isthmus. The ovary was normally developed and there was a normal growth and ovulation of yolks. However, as the yolks could not go through the oviduct, they passed into the abdominal cavity where they caused peritonitis. In a male, having genetic factors for this atresia isthmi, there was curiously shown a rupture of the dextral seminal duct just posterior to the testis. This defect, which in females has a typical sub-lethal effect, is assumed to be caused by a single dominant factor. In males the defect does not have a lethal effect. If only one of the seminal ducts is ruptured, it follows that the defect can be passed on to progeny through the male.

A somewhat similar condition has been described by Cole and Hutt (1953). These authors reported that of 324 non-layers, 8 per cent had incomplete oviducts. In most of these birds, the infundibulum portion of the oviduct persisted while a section from the magnum degenerated. In some cases the isthmus was also lacking. The ovary apparently functions normally and releases fully formed, normal ova, all of which are apparently resorbed soom after ovulation without having entered the oviduct. The birds appear normal in ovarian activity, in physiological changes in the external appearance of the body and in the internal organs and as well as in behavior. The proportion of non-layers in the population ranged from 0.3 to 2.9 per cent in four successive years. The authors believed that the differences among strains and sires may indicate genetic factors involved. Sturkie (1955) performed laparatomies in hens that appeared to be in productive condition but which were not actually laying. In most birds, the ovaries and oviducts appeared normal, and there were no ova in the body cavity, suggesting that ova were resorbed. Egg yolks were introduced into the body cavities of such birds and also of normal hens, and the incisions were closed. Twenty-four hours later, the birds were autopsied and inspected for the presence or absence of yolks. No trace of egg yolks was found in most of the birds indicating that yolks had been resorbed.

Hutt et al (1956) described several conditions of non-layers in a flock of 13,000 White Leghorn chickens in which he found a frequency of 1.38 per cent of non-layers at 13 to 15 months of age. The authors classified the different types of non-layers as follows: 1--ovary inactive; 2--ovarian retrogression; 3--ovulating non-layer; 4--oviduct discontinuous, cystic; 5--oviduct inpacted; 6--abdominal inclusions; 7--other conditions (polycystic ovary, undeveloped oviduct, cystic right oviduct). The highest frequency was found to be ovulating non-layers, discontinuous or cystic oviducts and ovarian retrogression. The frequency of ovulating non-layers was 4.4 per 1000. The authors found that most cystic oviducts are discontinuous, but that some are complete and closed at the anterior end. They indicated that the former type is probably congenital, though not necessarily genetic in origin, but that the latter can develop after laying has begun. The frequency of discontinuous oviducts was found to be 7.7 per 1000 pullets at 20 weeks of age. Mortality in these

birds from 5 to 8 months was more than twice as high as in others. The high frequency of ovarian retrogression was tentatively attributed to artificial lighting of immature pullets, with resultant refractoriness to light and disturbed endocrine balance at maturity.

Mason (1952) observed that adequate doses of progestrone in combination with low doses of estrogens caused hypertrophy of the oviduct, but that progesterone in combination with large doses of estrogens caused a decrease in oviduct weight. Decreased oviduct weight was observed when progesterone at 500 ug. was used with 200 ug. of estradiol or stilbestrol per day.

3. Factors affecting albumen secretion and/or albumen quality.

Biological processes are generally inhibited by ionizing radiation, except at low levels, and the biological response to the irradiations is proportional to the quantity of radiant energy absorbed by the tissue or organism. The mechanism of action of ionizing radiation is not yet well understood.

Smith et al. (1956) irradiated laying White Leghorn chickens by exposing the oviducts and surrounding them by lead plates so as to shield the rest of the organs from radiation. The birds were between 9 and 18 months of age. The radiation doses varied from 50 to 5,000 r. with a dosage rate of 425 r. per minute. The mean laying rate of all hens prior to irradiation was 69 per cent, the mean egg weight about 57 grams. Egg weight was significantly decreased at dosages of 3,000 r. or greater. Most of the decrease in egg weight resulted from a diminished albumen. The inhibition of albumen formation was found to increase by a factor of 2/3 with each 1000 r. increase in irradiation. A decrease in shell weight was noted in birds receiving 3,500 r. when the whole oviduct was irradiated. The irradiation of the oviduct did not seem to affect yolk weight, which was to be expected, since the ovary was well protected. This independence in yolk and albumen weight indicates that the weight of the albumen is largely determined by the oviduct characteristics, rather than by merely the degree of stimulation by yolk size. At levels above 4,000 r. very little thick albumen was evident. An abnormal appearance of the albumen was observed, resulting from opaque material occurring in large bodies or in dispersed form presenting a milky appearance. There was a large number of abnormalities in egg shape in all treatment groups. The recovery time following treatment was between 2 and 3 weeks.

4. Factors affecting the deposition of egg shell.

a) Sulfonamides.

A large amount of work has been carried out on the affect of sulfonilamide and related compounds on egg production.

Some of these drugs have been and are used for the treatment against blackhead disease in turkeys and pheasants. Hinshaw et al. (1943) observed that soft-shelled eggs were produced in turkey hens within several hours after insertion of a single dose of sulfonilamide in the crop. The single dose capsule contained about 16 mg. of the drug. Adult White Leghorn females given single doses of 48 to 80 mg. sulfonilamide showed the same results. The effect of this drug on shell thickness was confirmed by Scott et al. (1944) by feeding at levels between 0.5 and 0.002 per cent.

b) The role of carbonic anhydrase and its inhibition by sulfonamides.

Meldrum and Roughton (1933) were the first to advance the hypothesis that carbonic anhydrase might play a part in egg shell formation by influencing the rate of formation of the anion of calcium carbonate by catalysis of the reaction:

$$CO2 + H_2O \text{ -- } H_2CO_3$$

It was shown by Keilin and Mann (1940) that compounds of the RSO_2NH_2 type (where R is a benzene, naphthalene or pyridine ring) specifically inhibit carbonic anhydrase in very small concentrations. This property is absent in such compounds in which the sulfonamide group is substituted: like sulfopyridine, and sulfathiozole. This was confirmed by Benesh et al. (1944) and by Bernard and Genest (1945). The latter, however, observed that by feeding sulfapyridine, there was a decrease in shell thickness varying between 19 and 25 per cent. Although the authors did not rule out the possibility that the drug could affect carbonic anhydrase, they suggested that the effect may be in inhibiting other enzyme systems associated with shell formation. Gutowska and Mitchell (1945) observed that there is a relationship between the local concentration of carbonic anhydrase in the shell gland and the hen's ability to lay strong, smooth-shelled eggs. Carbonic Anhydrase acts as catalyst in the shell gland for the decomposition of carbonic acid, which may be formed from the bicarbonate ions in the blood, thus allowing a greater number of carbonate ions to be produced. Therefore, the enzyme does not act in the formation of carbonic acid as suggested by Meldrum and Roughton (1933).

The reaction:

$$2HCO_3 \rightarrow H_2CO_3 + CO_3$$

$$H_2CO_3 \xrightarrow[C.\ A.]{} H_2O + CO_2$$

The carbonate ions are then utilized in the formation of calcium carbonate, calcium being absorbed by the shell gland in its bound form (calcium proteinate) and dissociating it from the protein. Gutowska et al. (1945) inhibited the activity of

carbonic anhydrase by the subcutaneous administration of sulfanilamide at levels of 0.16 gms. / kg. of body weight, thus confirming the results of previous workers. Mehrring et al. (1955) confirmed the hypothesis that the SO_2NH_2 group of the drugs is the inhibiting factor of carbonic anhydrase, by feeding to New Hampshire chickens two unsubstituted sulfonamides, Diamox and benzenesulfonamide. Both compounds caused the pullets to lay eggs with very thin shells or no shells at all. The sulfonamide group is the only chemical portion these two compounds have in common.

c) The effects of Newcastle disease.

Berg et al. (1947) indicated that Newcastle disease markedly decreases egg production, albumen quality, and shell thickness and smoothness. The authors observed that the loss in albumen quality as a rule was permanent and that the decrease in shell thickness may be affected for several months. Clegg and Muller (1951) showed that hens apparently recovering from the infection produced poor shells, although showing the normal serum calcium level for laying hens. The authors concluded that the poor quality of the shell was due to malfunction of the uterus produced by direct effect upon this region or upon disturbances of factors controlling shell secretion. Biswall and Morrill (1954) observed the same effects of Newcastle disease and also pointed out that although the rest of the reproductive system is susceptible to the virus, the uterus is the only section to suffer functionally.

d) The effect of various other compounds.

Arasan: Arasan-SFX is a fungicidal agent used in treatment of seeds. It contains 75 per cent (T.M.T.D.) tetramethylthiuram disulfide. Swanson et al. (1956) feeding diets containing TMTD as low as 10 ppm. produced nearly 4 per cent eggs with very little shell or no shell at all. At levels of 100 ppm. no hard-shelled eggs were produced. Misshapen eggs were also observed at levels of 20 to 50 ppm. Recovery was complete after 2 days from withdrawal of the treated feed.

5. Factors producing delayed or premature oviposition.

Burrows and Byerly (1942) observed that intravenous injections of 2 units of obstetricial pituitrin in Rhode Island Red hens caused the premature expulsion of the eggs at any time after the egg had entered the uterus, but that eggs at higher oviducal levels were not laid prematurely.

Sturkie and Weiss (1950) found that by intramuscularly injecting acetylcholine (10 mg. or higher) into hens which had

eggs in the uterus, premature oviposition of from 2 to 18 hours in approximately 50 per cent of the birds was induced. These eggs were either soft-shelled or had measurably reduced shell content. Eggs from birds injected repeatedly with acetylcholine, showed decreased volume and increased viscosity of the thin white.

Ephedrine sulfate on the other hand delayed oviposition up to 24 hours when injected intramuscularly at the time the egg was in the uterus. Weiss and Sturkie (1952) confirmed their previous work on the effect of acetylcholine and ephedrine on influencing premature oviposition and delayed oviposition, respectively. The dose administered to 2-year old White Leghorn chickens were between 50 and 100 mg. of acetylcholine and 25 mg. or larger amounts of Ephedrine. These workers observed that histamine will also produce premature oviposition with a total dose of 15 mg. The amount of shell found on the eggs varied depending on the time the eggs had spent in the uterus before the drug was injected.

Sykes (1955) delayed oviposition in Brown Leghorn chickens by administration of 1.0 mg. or more adrenaline. The author suggested that the most likely site of action of the drug would be the uterine muscle. Oviposition took place after injecting the birds with pituitrin. The doses that were too small to delay oviposition were nevertheless effective in lowering production. Inhibition of ovulation and follicular atresia have been caused by fright, which in mammals is known to promote the release of adrenaline -- and the same may be true of the fowl. Adrenaline may act directly on the follicles or through the pituitary gland.

6. Shell structure as affecting fertility or hatchability.

Pritsker (1941) reported that up to 50 per cent of duck eggs occur with a glossy "fatty" surface. Chemical analysis of shells of fatty and non-fatty eggs showed large differences in composition. In shells with fatty deposits, the protein had been supplanted by fats to the extent of 70 per cent, fats contributing over 80 per cent of the total quantity of organic matter. In studies carried out to find the effect on hatchability, it was found that the per cent of dead germs up to 7 days was 15 per cent in the fatty eggs as compared to 5 per cent in non-fatty eggs; between 7 and 21 days, it was 28 and 10 per cent, respectively. The per cent fertile eggs that hatched was 23 per cent for fatty eggs as compared to 52 per cent for non-fatty eggs. The per cent of strong ducklings was greatly reduced also. Pritsker believed that the presence of the "fatty" deposits on the shells of ducks is not the cause of the high death rate of embryos, but a sign of more vital changes within the eggs themselves.

The amount of pigment deposited in the shell seems to be positively correlated with hatchability. Godfrey (1947) observed that in New Hampshire chickens and White Plymouth Rock chickens extremely light-colored eggs hatched poorly in comparison with medium and dark brown ones. This observation was corroborated by Funk and Forward (1949) in New Hampshire chickens, who found the per cent hatchability of dark brown, medium and light brown eggs to be 74.1, 70.0 and 55.0 per cent respectively. Embryonic mortality during the first 18 days of incubation and also during the hatching period was greater in light brown eggs.

The rate of evaporation also reduced hatchability in most cases; evaporation is regulated by the shell texture or porosity of the shell. Hays and Spear (1951) found in experiments with eggs of Rhode Island Red chickens that the weight loss to 17 days of incubation varied between 6.5 and 12 per cent without affecting hatchability; losses greater than 12 per cent lowered hatchability considerably. The hatchability obtained in his experiments was 71 to 92 per cent with losses between 6.5 and 12 per cent, and 49 to 67 per cent with losses between 13 and 18 per cent.

Rauch (1952) showed that the mean pore diameter in shells of eggs producing hatched chicks was less than in shells from non-hatching eggs. The pore size for shells of hatched chicks was between 9.4 and 10.1 u.,those for shells of non-hatching chicks was between 12.0 and 21.8 u.

7. Miscellaneous factors.

Olsen and Haynes (1949) conducted studies on freshly laid eggs to determine the effect of certain abnormal characteristics on fertility and hatchability. Four per cent of the 48,000 eggs had one of the following characteristics. The hatchability of fertile eggs showing these characteristics was as follows: small eggs, 80.0 per cent; large blood spots, 71.5 per cent; extra large eggs, 70.8 per cent; misplaced air cells, 68.1 per cent; misshapen eggs, 48.9 per cent; cracked eggs, 53.2 per cent; eggs with poor shells, 47.3 per cent; loose air cells, 32.4 per cent. The average hatchability for reference control eggs was 87.2 per cent.

Fertility and hatchability may be affected by substances toxic to the embryo entering into the components of the egg. Polin *et al.* (1961) found that the concentration of Glycarbylamide, a medicant used for the prevention of coccidiosis in chickens, in the egg white increased with increasing levels of the drug in the feed until a maximum concentration averaging 12.9 ug. per gram at dietary levels of 0.10 per cent. The drug was fed to 10 White Leghorn females and to one rooster, at levels between .02 and 0.16 per cent during 21 days. Concentration of 0.08 and 0.16 caused a significant decrease in

fertility (48 and 24 per cent, respectively). Significant differences in hatchability were also observed at these levels. Glycarbylamide did not have any effect on the fertility of the rooster. The authors believed that the sperm in the oviduct of medicated hens may be killed by contact with glycarbylamide in the albumen lining the lumen of the magnum or that the drug imparts a gametocidal effect directly on the hen. The birds returned to normal after four days from the removal of the drug.

Other factors may affect the composition or characteristics of the laid eggs, thus affecting directly or indirectly fertility, hatchability and the subsequent performance of the mature bird. Some components of the rations affect the formation of the egg more than others. It should be mentioned that the level of calcium, phosphorus and vitamin D in the diet affect to different degrees not only egg production in general but also the overall amounts of these components in the egg affecting hatchability in the long run.

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RECENT FINDINGS IN THE INHIBITION OF AVIAN EMBRYOGENESIS

Macreay J. Landy

1. Normal sequence of embryogenesis.

2. Genetic lethal factors.

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RECENT FINDINGS IN THE INHIBITION OF AVIAN EMBRYOGENESIS

Macreay J. Landy

1. Normal sequence of embryogenesis.

Embryogenesis is in progress as the zygote moves down the oviduct and finally appears on the outside. Fertilization usually takes place in the infundibulum of the female as was first noted in studies with the pigeon (Patterson, 1910). However, fertilization may take place as soon as the germinal disc is exposed by rupture of the follicular wall. Normally 15 minutes elapses between ovulation and the entrance of the sperm (Romanoff, 1960).

As the sperm and egg come into close proximity, various substances recognized only as fertilizins, antifertilizins, agglutinators, etc. are secreted by sperm and egg alike. These substances have been studied extensively in the sea urchin. These substances interact to inhibit or facilitate sperm penetration.

The vitelline membrane surrounding the ovum is penetrated by the sperm or by many sperms. As many as 25 separate sperm have been counted in the vitelline membrane (Harper, 1904). The sperm enters the germinal disc and the second maturation division of the egg nucleus proceeds to completion. A reduction division occurs with the formation of the second polar body, and either at this time or at the second maturation division metaphase stage, fertilization is said to take place.

The male and female pronuclei are formed. As the zygote travels down the oviduct carried by peristaltic movements, the formation of the segmentation nucleus takes place. Here, in the oviduct, the ovum is invested with several layers of albumen. The zygote then travels to the isthmus where the shell membrane is laid down. In this area, the first segmentation division takes place. Next, the calcareous shell is secreted around the zygote. Meanwhile, the development has proceeded to the multicellular stage with the formation of the secondary germinal layer during cleavage.

Gametogenesis and fertilization are necessary precursors to embryogenesis. At any point during this sequence, we might interrupt or interfere with the processes going on and thus prevent or delay the next step.

At the sperm-egg interaction level, the structure of the ovum is important. It is at this stage that there is an interaction of substances termed fertilizins, lytic agents, etc. In the sea urchin and the frog, these processes are an antigen-antibody type reaction.

As the egg enters the oviduct, a series of divisions is proceeding at a rapid rate. Nelson and Segal (1958) working with rats found that a triphenyl ethanol derivative MER-25 prevented the further development of the zygote as it moved down the oviduct. Twenty-five mg. per kilo body weight was given orally to female rats for 4 days after the first sperm was detected in the vagina. Implantation was prevented in all cases. The substance is believed to act rapidly on the ova after the extrusion of the second polar body, and it is believed to be secreted directly into the lumen of the oviduct and there act directly on the egg. MER-25 was found to have no effect after implantation.

Another stage at which inhibition has been demonstrated, again not in avian species, is the blastula stage of development. Brachet (1958) demonstrated that B-mercaptoethanol inhibited cleavage by inhibiting cell division. The compound is suspected to act as a reducing substance on the -SH linkages of the amino acids involved in the protein build-up necessary in cell division. Embryogenesis has been described chiefly by Weiss (1948). "An embryo is built like an automobile, on an assembly line by innumerable processes succeeding each other--some with a latitude for repairs, others not. At any one point along that complicated line, some agent which we apply may strike. Sometimes it strikes at a time we apply it and sometimes later, because of a latency of its actions."

Inhibition is described as the restraining of, or the cessation of, or the interference with, the function of an organ or agent. This may be at a specific tissue or cellular area or at some more general area. At any time in the developmental stages of the chick from the embryo to the mature chicken, the expression of lethal genes may take place. Here, the definition of embryogenesis is somewhat extended to take in the delayed action lethal recessive.

2. Genetic lethal factors.

The first lethal mutation in poultry was reported by Dunn (1923). The Wyandotte lethal as it was termed was found to reduce hatchability to 30 per cent. Since then, a wide range of lethals has been reported which have been summarized by Landauer (1951). Subsequently, several new lethal mutations have been reported.

Bernier (1953) reported a dominant lethal in Single Comb White Leghorns. This is an obligate lethal which affects the chicken at 1 to 4 days; circulation is not properly established.

The Sparrow Head lethal was reported in New Hampshire chickens by Gyles, Gilbreath and Smith (1953). This is a delayed lethal recessive which produces a distinct shortening of the head bones and beak at 9 weeks to maturity.

Hereditary exencephaly was reported by Mellen in 1954 as a recessive facultative lethal which produces a wide range of effects from slight hyperencephalocele to a massive herniation of the brain.

Cyclocephaly and cyclopia lethals were reported by Landauer in 1956. Wetherbee (1958) described microphthalmia in Quiscalus quiscula and synophthalmia in Mimus polyglottos. Other lethals recently described are "dehydrated" by Taylor and Stinnett (1956); Second Diplopod by Landauer (1956); Paroxysm by Cole (1961); and Congenital loco by Cole (1956).

The same syndrome of effects may be produced in a given species by entirely independent recessive or dominant mutations. An example of this is the various forms of micromelia or rumplessness reported as a dominant by Dunn (1925) and as a recessive by Landauer, 1945. Extragenetic factors such as season and environment may act to modify the gene expression. Then, too, penetrance (incidence of affected individuals) and expressivity (the degree of the effect) may be altered by genetic and environmental factors.

In summary, then, the lethal and teratogenetic effects of hereditary factors are many and varied. Not all are the result of a dominant mutations, but may be dominant or recessive and facultative or obligate. All act to inhibit embryogenesis in some way.

The discovery of genetic lethals has been accompanied by a corresponding study in the mechanisms of gene action. Experimentally, chemical teratogens have been employed that have effects similar to genetic teratogens.

3. Phenocopies.

A normal embryo can be modified in its development so that the end product "duplicates" or closely resembles known mutant traits of the same species. This phenomenon has been termed phenocopy by Goldsmidt, (1935). Landauer (1959) warns that this term should be limited "to external stimuli or sources of modification which exert or cause identical action on the embryo (similar phenotypic expression) without vital invasion and destruction of certain embryonic organs and tissues."

One of the most frequently studied abnormalities is that of micromelia. During the interval from 4 to 6 days of age, the embryo is especially sensitive to a host of compounds which can induce this response. Landauer (1954) has summarized the typical symptoms produced in the chick embryos by these teratogenic substances and his summary is reproduced here in a somewhat modified table form. (See table #1).

Table I (Adapted from Landauer, 1954)

Typical Symptoms of Chick Embryos Produced by Teratogenic Substances Applied during Period of Micromelia Response

Teratogen	Facial Skeleton	Long Bones of Leg	Toes	Other Defects
Sulfanilamide	Parrot beak	Micromelia, bending of tibiotarsus	Syndactylism	Clubbed down
Eserine	Parrot beak	Micromelia, bending of tibiotarsus	Syndactylism	Clubbed down
Insulin	Short upper or parrot beak, rare facial coloboma	Micromelia, maximal shortness and bending of tibiotarsus	Syndactylism low incidence	Microphthalmia, buphthalmia, coloboma of upper lid
Boric acid	Short low beak, facial coloboma, cleft palate	Micromelia, maximal shortness and bending of tarsometatarsus	Syndactylism, shortening of lack of toes, preferentially the 4th	Curled toe paralysis
Pilocarpine	Depends on dose Short upper and parrot beak, short lower beak, facial coloboma and cleft palate	Micromelia, maximal shortness and bending of tarsometatarsus	Syndactylism, shortening or lack of 1st toe	Clubbed down, microphthalmia, coloboma of upper lid
Thallium	Parrot beak	Micromelia, bending of femur and ribiotarsus	------	Microphthalmia

Table 2 (Adapted from Landauer, 1954)

Relative Dose and Incidence of Resulting Malformations at 96 Hours

		Beak Defects %		Abnormalities of Long Bones %	
Insulin (units)	2	16.7	3.17	26.8	3.75
	5	55.0	4.97	80.0	4.00
Boric acid (mg)	1	0.5		0	
	2,5	69.9		17.6	
	3.5	85.3		39.7	
Pilocarpine Hydrochloride (mg)	3	0.9		0.6	
	6	12.0		29.9	
	12	41.8		83.5	

Neuman, et al. (1956) injected White Leghorns at 96 hours of incubation with varying amounts of semi-carbazide. Doses in excess of 3 mg. killed the embryo. Doses of 2.3 mg. at 4 days caused shortened and malformed lower beak, and a bending of the tarsometatarsal and tibiotarsal bones. The suspected mode of action was an inhibition of diamine oxidase and perhaps other enzymes responsible for deamination. The resulting effects were relative to the doses and the developmental stage at which the drug was administered. A summary of these experiments is reproduced in Table #2.

Landauer (1953) demonstrated a relationship between the stage of development at which the dose was delivered and the resulting effect. Insulin administered at 84,120 and 160 hours in doses of 2 units yielded an over-all effect of a uniform shortening of the embryo. However, the effects were greater when administered at the earlier stages of development. Syndactylism was produced in greatest incidence at 84 hours.

Neuman (1956) demonstrated that the administration of semi-carbazide was subject to "a sensitive stage of development (after which a decreasing number of lethalities and abnormalities were produced". Administration yielded distortion and irregularities with twisted legs and beaks. If administered at 12 days, the embryo developed normally.

4. Other chemical factors.

There are other chemical inhibitory agents the effects of which have not been linked to mutant gene expression either because of lack of knowledge of such a gene or because the chemical acts upon the cells directly. Among these are compounds classified as antimetabolites which can be further subdivided according to their suspected mode of action either as analogues of purines, or of amino acids, or of vitamins.

Waddington (1958) and Friar and Woodside (1956) wound that 8-azaquanine when injected into the egg caused retardation of the whole embryo, especially of the brain and eye cups, a retardation of the somite formation and the lack of formation of well-defined blood vessels. The chemical varied in its effects at various developmental stages and according to the relative amounts administered. Both azahypoxamine and 8-azaquanine at 0.1 per cent arrested cells in early prophase. Benzimidazole at 18 hours and 0.5 per cent produced slight cellular degeneration in the newly formed mesoderm and an inhibition of the head region growth.

Another purine analogue, L-azaserine, when injected (0.20 mg.) into 4-day-old embryos produced skeletal abnormalities, eye defects, and three types of beak defects Dagg (1955). Twelve-day embryos showed no skeletal defects when injected with the same amount. LD_{50} studies showed that the dose tolerance

Classification of some candidate materials inhibiting embryogenesis

Analogues act with one or several definite chemical components of the cell. Due to their close resemblance structurally to vitamins (metabolic intermediaries) they interfere specifically with the proper function of the corresponding natural substances

Purine analogues

8-azaquanine (Waddington, 1955)
azahypoxamine "
benzimidazole "
L-azaserine (Blattner, 1958; Dagg, 1955)
quanazole (Younger et al., 1950)

Amino acid analogues

ethionine (Feldman & Waddington, 1955)
α -NH_2-β - chloro-valeric acid (Herrman, 1953)
DL-ω- bromoallylglycerine (Herrman, 1953)
α -NH- β -chloro-butyric acid (Herrman, 1953)
β- 2 - thienylalanine (Herrman, 1953)
para-chlorophenylalanine (Herrman, 1953)
ortho-chlorophenylalanine (Herrman, 1953)
ω -bromoallylglycine (-(Herrman, 1955)
α -bromoallylglycine (-(Herrman, 1955)
propargyl glycine (-(Herrman, 1955)
cyclopentaneglycine (-(Herrman, 1955)
DL-bromopropionylalanine (-(Herrman, 1955)

Aliphatic acid analogues (-(Rothfels, 1954)

Aromatic acid analogues (-(Rothfels, 1954)

β -2-thienylalanine (-(Rothfels, 1954)
p-O-chloro-phenylalanine (-(Rothfels, 1954)

Other analogues

benzimidazoles (-(Blackwood & Shorb, 1958)
2-ethyl-5-methylbenzimidozole (-(Blackwood & Shorb, 1958)
2-hepta-5-methylbenzimidozole (-(Blackwood & Shorb, 1958)
2-ethyl-5-methylbenzimidozole (-(Blackwood & Shorb, 1958)
(also inhibits biosynthetic processes involving nucleic acid or nucleoprotein build-up) (-(Blackwood & Shorb, 1958)

Steroids

cortisone (Sobel, 1958)
sex hormones (sunthetic and extracted)
testosterone propionate
diethylstilbesterol (van Tienhoven 1957)

Goitrogens

Thiourea (Sinha, 1959)
Thiouracil "
Thyroxine (Beyer, 1952)

Inhibitors of Tissue Differentiation

Diazo dyes (Wilson, 1955; Beaudoin, 1961)
Trypan Blue "
Evans Blue "
Niagara Blue 4B "

Mitotic Interference

triethanomelamine TEM (Jurand, 1958, 59, 1960, 61)
fluro-acetyl derivative of "
N-(p-amino-phenyl)-nitrogen mustard "

Hatchability Suppressor

Sterculia foetida oil (suspected
Crude cottonseed oil (reactive cyclopropane fatty acids

increased with the age of the embryo, from 0.10 mg. at 0 to 2 days to 2.40 mg. injected at 12 days. Blattner et al. (1958) inhibited hatching by injecting 0.5 mg. into the blastoderm region of the egg. The probable mode of action is interference with adenine metabolism.

Quanazolo was tested on White Leghorn chickens by Younger et al. (1950). The general effects noted were a reduction of vascularization and of development of the chorioallantoic membrane, abnormalities of the beak and inhibition of feather formation. A dose-effect response was noted with the percentage mortality decreasing with decrease in doze (0.5 mg., 100 per cent mortality; 0.06 mg., 43 per cent mortality). Sparing-reaction studies demonstrated that the purine affected was probably quanine.

Amino acid analogues have also been tested for possible inhibitory action. Among those found to be most effective through direct application to the embryo either in the egg or as an explant were derivatives of analine and glycine. Herrman (1953) treated explanted chick embryos at the 6 somite stage with the following analogues. B-2 thienylananine and parachlorophenylalanine were found to produce the most extensive abnormalities and to increase the mortality. A general retardation of growth, a zigzag neural tube and an irregularly formed brain were the most common abnormalities produced. Two aliphatic amino acids did not have as pronounced effects on the central nervous system as did the above mentioned benzenoid compounds. They did, however, produce a general inhibition of growth.

Five other derivatives of alanine and glycine, antagonists of leucine and methionine, were tested by Herrman (1955) on chick explants. Injection into the yolk sac and onto the chorioallantoic membrane of x-bromoallyglycine and w-bromoallylglycerine produced suppression of somite segregation at dose levels of 0.1 mg. Increased effects were found to occur at higher concentrations.

The mode of action for all the foregoing compounds as suggested by their structural resemblance to amino acids, was probably that of interference with protein synthesis.

Herrman (1953,1955) and Feldman and Waddington (1955) demonstrated that the aliphatic amino acids caused the failure of segmentation of the mesoderm, hence the appearance of "blocks of unsegmented somites". The aromatic amino acids produced retarded growth, increased percentage of abnormalities and increased percentage mortality.

Another fertile field of investigation has been the vitamin analogues and nucleic acid antagonists. Blackwood and Shorb (1958) studied certain benzimidazoles and benezenes some of which were natural moieties of vitamin B_{12}. Two strains of

White Leghorn chickens were treated by injection into the albumin. Those compounds that produced the greatest mortality were not the natural moieties of vitamin B_{12}. Mortality varied with breed and season. The order in which the per cent mortality of the benzimidazoles occurred was much the same as that demonstrated for certain viruses, whereas the benezenes followed an inhibition pattern similar to that demonstrated for microorganisms which synthesize their own B_{12} and/or riboflavins.

The following steroids have been demonstrated to have adverse effects on the embryo only at the later stages in development, on or about the eighth day of incubation. Karnofsky (1951) stated that cortisone acted on the chorioallantoic membrane, thereby inhibiting growth and development. One mg. per egg produced a high mortality in the embryos tested. Danowski (1951) demonstrated that cortisone treated chick embryos excreted an excess of NaCl and reducing sugars into the allantoic fluid. He postulated that a negative electrolyte balance may play a part in producing the syndrome of the "corisone effect". Evans (1953) and Sobel (1958) found that one mg. of cotisone injected at 8 days produced effects on the liver, gonads, eyes and feather growth. The greatest inhibition occurred between the eighth and tenth days.

van Tienhoven (1957) dipped eggs in 5 mg. of diethylstilbesterol in 100 cc. of oil and in testosterone proprionate solution, 650 mg. T.P. in 100 cc. of ethanol. He found that hatchability was reduced 10 to 15 per cent with both treatments.

Certain chemical agents interfere with the synthesis of hormones. Thiourea and thiouracil are two such compounds. These compounds prevent the iodination of tyrosine and the production of thyroxine which are necessary for general growth and metabolism. Thiourea and thiouracil injected into the yolk sac of White Plymouth Rock chickens were found to have moderate yet important effects upon the embryo (Adams and Bull, 1949, and Grossowicz, 1946). At the eighth, fourteenth and eighteenth days of incubation, injections of thiourea and thiouracil produced an increase in the incubation period, lack of retraction of the yolk sac, decreased trunk and limb growth and obvious enlargement of the thyroid gland. Romanoff and Laufer (1956) demonstrated that there is a difference in the effects produced in the embryo before and after for the thyroid comes into function on the eleventh day (Hansborough and Khan, 1951). One of the effects, inhibition of gain in body weight, occurs only after the time of thyroid formation (Sinha, 1959).

Compounds other than those of metabolic importance to the embryo have been tested as possible teratogens and inhibitors. Diazo dyes such as Trypan blue and Evans blue were found to have pronounced effects on rats and chickens (Wilson, 1955).

Dyes were injected into the yolk sac and into the subgerminal cavity of White Leghorn embryos (Beaudoin, 1961). The effects are summarized in Table #3.

Table 3. (Adapted from Wilson, 1955)

Effects of dyes upon chicken embryos as expressed in per cent mortality and per cent malformations.

	Total Treated		% Mortality		% Malformed	
	Sub-germinal	yolk sac	Sub-germinal	yolk sac	Sub-germinal	yolk sac
Trypan Blue	101	94	45.5	56.5	72.8	56.2
Evans Blue	78	90	44.8	14.4	51.3	5.2
Niagara Blue (4B)	94	93	69.3	29.9	34.5	33.4
Saline controls	220	141	20.9	12.0	20.1	2.4
Untreated	--	134	---	11.9	---	3.4

Trypan blue was the most effective inhibitor at both of the injection sites. Evans blue and Niagara blue 4B were most effective when injected subgerminally. The two most frequent types of malformations produced were rumplessness and eye defects such as microphthalmia. A difference between the effects produced and the site of injection was noted. In general, yolk sac injections were less traumatic regardless of the material injected, as fewer deaths and abnormalities occurred from the injection itself. Possibly, as was suggested by Landauer and Bauman (1943), injection at other sites induced some sort of mechanical injury to the blastoderm.

Several drugs found useful in cancer research as mitotic inhibitors, have been used in studies with chick embryos. Among these is T.E.M. (triethylenemelamine) a nitrogen mustard derivative. Jurand (1958) demonstrated that T.E.M. in saline solution produced drastic cellular effects on the primitive streak stage embryo at very low doses. The mode of action, if similar to that of nitrogen mustard, might be chromosomal breakage with consequent chromosomal rearrangements, hence interruption of the mitotic process.

Our final consideration of chemical agents which produce inhibition is concerned with feed supplements. Ringnose, Morgan, and Lease (1941) described experiments in which crude cottonseed oil added to a practical laying ration reduced the hatchability of eggs produced. Naber and Morgan (1957) supplemented a mash with a 5 per cent solution of a hydraulic processed crude cottonseed oil. The result was a reduction in hatchability from 90 per cent to 6 per cent. Most of the mortality occurred at the fourth day, the next greatest mortality at the eighteenth day. A disruption of the extra embryonic circulatory system was noted in the early stages. In the late stages, hemorrhages occurred in the blood vessels.

Another oil, an extract of the nut Sterculi foetida, was added as a supplement to the feed by Schneider et al. (1961). 150 mg. of oil in a gel capsule when added as a supplement to the daily feed produced complete suppression of hatchability. Of the 23 eggs tested, 80 per cent were dead by the fifth day, 93 per cent by the tenth day, and 100 per cent by the nineteenth day. Lower concentrations of the oil caused a reduced hatchability. It was suspected that a Halphen reactive cyclopropene fatty acid was the hatch depressing factor as other components such as Sterculic acid when tested alone did not produce mortality.

5. Environmental factors.

The optimal temperature for incubation is 38.5 degrees C. for the chicken. Low temperatures limit development. The blood system fails to differentiate at 27 degrees C. (Funk and Biellier, 1944). At temperatures lower than 27 degrees C., the blastoderm fails to develop beyond the primitive streak stage (Harrison and Klein, 1954). Eggs subjected to 25 degrees C. have embryonic growth rate thirty times slower than at optimum temperature. Ten days was the time limit an egg could be held at 25 degrees C. and then put into optimum temperature (38.5 degrees C.) and have normal development.

High temperatures, on the other hand, increased the number of anomalies and the percentage mortality (Deuchar, 1952). Ancel (1958) demonstrated that any interruption of the normal incubation-development rate for two to three days resulted in an increase in mortality and teratogenesis. Further, the stage at which cooling takes place determined the numbers and kinds of malformation or deaths.

Dietary deficiencies of laying hens can be responsible for anomalies in the developing young. Landauer (1951) and Cravens (1952) have summarized the role of vitamin deficiencies in the laying hen upon the developing embryo. Most important of the vitamins are biotin, nicotinamide, vitamin B_6 and Folic acid.

New (1957) demonstrated the necessity of turning eggs during artificial incubation. The critical period was found to be 4 to 7 days.

Irradiation by x-ray or gamma nuclear radiation affects hatchability and fertility. Glasser et al. (1955) noted that total dose and percentage survival showed an inverse relationship for both 6- and 12-day-old embryos irradiated. At 20 r. per minute, 400 r. produced 96.9 per cent survival, while 800 r. produced 0.7 per cent survival. Goldman found that 800 r. at 17r. per minute killed 62 per cent of irradiated eggs within 21 hours and 100 per cent at 48 hours. In the wild, Norris (1958) irradiated nine eggs of the Eastern Bluebird. The mean dose was 420 r. The results were 3 embryos dead, 3 hatchlings dead and 3 fledged. When adults were treated with radiation and their fledgling success compared with untreated adults, no significant difference was noted at doses of 200 r. and 600 r.

Irradiation dosimetry and numbers of experimental subjects constitute the greatest weakness of work done in this area. Currently, Wetherbee and Landy are conducting experiments in irradiation at the University of Massachusetts.

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RECENT FINDINGS IN THE INHIBITION OF AVIAN OOGENESIS

Ralph G. Somes, Jr.

1. Normal sequence of oogenesis.

2. Inhibition of egg production.

3. Additives and deficiencies in yolk formation, detrimental to hatchability.

4. Inhibition of germinal viability resulting in infertile eggs.

5. Literature cited.

RECENT FINDINGS IN THE INHIBITION OF AVIAN OOGENESIS

Ralph G. Somes, Jr.

1. Normal sequence of oogenesis.

Oogenesis in the chick begins very shortly following fertilization. According to Romanoff (1960) the primordial germ cells can be recognized as early as 18 hours incubation, when they are still situated anterior and anterolateral to the embryo proper. This region is referred to as the "germinal crescent". According to Blocker, as stated by Romanoff, the germ cells become intermingled with the blood cells of the developing blood islands as the mesoderm moves forward between the ectoderm and the endoderm and surrounds the germ cells. At about 33 hours of incubation, the embryonic circulatory system is continuous and the germ cells enter the embryo's body and migrate, by 55 hours, to the gonadal regions. The germ cells distribute themselves very unequally between the two sides of the embryo with the left side receiving many more than the right. Between the eighth and eleventh days, the germ cells divide rapidly and decrease in size. They are then known as oogonia. During the 12th to 14th days, the cytoplasm increases and the enlarged oogonia become primary oocytes. Very little growth occurs between now and hatching, but between the 15th and 21st days, the nucleus goes through the first meiosis division. Following hatching, follicle formation begins along with white yolk accumulation. The ova increase in size until they are about 4 mm. in diameter. At two to three months of age, the second meiosis division begins, but it is not completed until the ova is fertilized following ovulation. At 5 to 9 days before ovulation, according to Marza and Marza, (Romanoff, 1960), the ova's growth rate accelerates rapidly by the addition of yellow yolk. By the time of ovulation, the ovum has usually attained a diameter of 35 mm.

Ovulation itself is under hormonal control as is the yolk accumulation just mentioned. Fraps and co-workers, as stated by Sturkie (1954), have demonstrated that ovulation is induced by the cyclic release of LH (luteinizing hormone) from the pituitary. This hormone is released by the pituitary 6 to 8 hours prior to ovulation. As long as an egg is in the oviduct, further ovulation is inhibited, but upon oviposition another egg is usually ovulated within 15 to 75 minutes in the chicken and 4 to 5 hours in the pigeon according to Sturkie (1954).

Birds generally lay their eggs in cycles or clutches which can vary from one egg as with the auks and penguins to as many as 12 to 20 in partridges, (Romanoff and Romanoff, 1949). Removal of eggs from the nest may prolong egg laying in many wild birds and removal contributes to the year-round laying of the domestic hen. Booker and Sturkie (1950) have suggested that possibly thyroxine secretion rate may play some part in the role

of determination of clutch length, but not the interval between clutches. They showed in hens that 4-egg cycle birds had larger thyroids and higher secretion rates than did 2-egg cycle birds.

2. Inhibition of egg production.

For reviews of inhibition prior to 1950, the following works are helpful: Sturkie Avian Physiology, 1954; Cole and Cupps Reproduction in Domestic Animals, 1959; Romanoff and Romanoff The Avian Egg, 1949 and Romanoff The Avian Embryo, 1960.

The two basic measurements of the success of oogenesis inhibition are per cent egg production and per cent hatchability resulting from a treatment as compared with a reference control. It is appropriate to first consider a paper by Titus (1953) on the size differences in these two measurements necessary for significant difference. Titus ran a "uniformity trial" with 600 Rhode Island Red pullets which had not been pretested or selected for uniformity. He found the coefficient of variation of the number of eggs layed to be between 17 per cent and 25 per cent with a modal value of 20 per cent. For hatchability, the coefficient of variation was 12 per cent to 30 per cent with a 20 per cent modal value. Therefore, he estimated the number of birds needed to show a difference in egg production of 10 eggs when average egg production was 200 eggs and a difference in hatchability of 5 per cent when the average was 70 per cent (at the 5 per cent level of significance using the modal coefficients of variation) would have to be not less than 123 birds for egg production and 60 for per cent hatchability. The use of the smallest coefficient of variation requires not less than 94 birds for egg production and not less than 24 for per cent hatchability. On the basis of these criteria, the conclusions of some of the following papers might well be questioned.

Egg production as a physiological process is influenced by a number of external environmental factors. The literature on these, particularly on light, is voluminous enough to be a seminar in itself and, therefore, I shall not dwell on these, other than to mention them. They are not generally adapted to manipulation as far as wild birds are concerned. A review by Marshall (1959) covers a number of these.

As the work of numerous workers has shown, light stimulates egg production; lack of it or too much of it may have an inhibitory effect. However, some species particularly those in dry areas and the tropical regions may not be directly affected by light regulation.

Temperature and rainfall are two important factors for some bird species. Sudden drops in temperature or extremely high

temperature generally inhibits ovulation, while in Western Australia, which is relatively dry, rainfall stimulates egg production in some species.

Other external factors that affect the beginning of egg production are: establishment of territory, availability of nesting site and building materials, abundant food supplies and for some bird species the necessity of being a member of a large breeding colony. Egg production is inherited in chickens as it probably is in most species; in poultry it is an extremely complex characteristic involving many genes;

Briles (1956, 1960, 1961) has found a relationship between blood types and egg production. Over a period of three seasons, he found that the hens in two lines heterozygous for B blood group alleles were superior to the homozygotes. On a survivor basis, in one of these lines the heterozygotes were 4.5 per cent better and in the other line they were 6.9 per cent better egg producers.

More recently, work at this station (Smyth, 1961) has shown a definite correlation between the degree of melanization of laying hens and their per cent egg production. The lighter birds had the highest production and the darkest birds had the poorest production. Melanization groups between these extremes fell right in line with the correlation.

Now to consider some of the hormone manipulations of egg production-- A number of studies have dealt with the treatment of hens, their eggs or the developing pullets to determine the effects on reproduction. Adams et al. (1950) reported abnormal reproduction from pullets which had developed from eggs from hens which had been fed various levels, ranging from 0.01 per cent to 0.04 per cent of two synthetic estrogens. Glick (1961), using very small numbers reported the results of dipping eggs of three days incubation in 100 ml. solutions of ethyl alcohol, 670 mg. testosterone propionate, 2 gm. testosterone propionate and 670 mg. dehydroisoandrosterone. Production for the resulting pullets in the testosterone groups was lowered by 16 per cent. The hens produced no fertile eggs in the 2 gm. testosterone group and hatchability was lowered by 14 per cent in the 670 mg. testosterone group.

Estrogens given to 9-week-old or older birds, either in the feed at .04 per cent level (Adams et al., 1950), or as a 12 to 15 mg. diethylstilbestrol implanted pellet in chickens (Akpinar et al., 1953; Talmadge, 1959; Jeannout et al., 1959), or as a 24 mg. pellet in turkeys, or as an intramuscular injection at the rate of 4.16 mg./week (Herrick et al., 1955), all produced a marked depression in egg production. In most cases, the depression effect lasted only during the first three to four months of production.

Large injections of progesterone have been shown to completely and immediately stop egg production followed in 10 to 14 days by a rapid molt (Herrick et al., 1955). The molt ceased 2 weeks after final injection and egg production resumed in 25 ± 2.6 days after final injection according to Adams (1955). Cook and Warnick (1961) classified a group of pullets into four production levels on the basis of past production. They then gave these birds various levels of progesterone for two weeks and observed them for twenty weeks after treatment. The injections were given every third day at the following levels. The production levels during the two weeks of treatment and after treatment for four weeks are listed.

Treatment:	0	2	4	8	16	32	64mg.
Treatment production:	10.2	10.5	6.9	4.2	2.1	2.9	2.4Eggs
4-week post treat. prod.:	18.2	19.4	15.0	15.9	9.5	9.0	8.3Eggs

After the four-week post-treatment period, production was the same in all groups. There was a linear response of production groups over all levels of progesterone, with high production groups showing less inhibition. This would suggest a possible genetic difference in resistance to gonadotropic inhibition.

The physiological basis for progesterone hormonal depression on egg production is presumably through its influence on the pituitary as a depressor of gonadotropic hormone secretion rate. Herrick and Adams (1955) showed that progesterone had the least lasting effect, followed by testosterone and lastly diethystilbestrol.

One other group of estrogen treatment studies should be mentioned here. This work seems to have a definite effect on sex ratio, although the physiological basis is not clear. Fraps and co-workers in 1956 injected 9-week old pullets with either 1, 2, 4, or 8 pellets of 15 mg. of diethylstilbestrol. The numbers of chicks that developed from eggs from these treated hens were found to be definitely heavy on the female side during the fourth and fifth months of production, but not after that. In a hatch of more than 30 chicks, the ratio was 40 per cent males and 60 per cent females. In another hatch of 67 chicks, the ratio was 42 per cent males and 58 per cent females. In 1958, Pun did a similar experiment with 34 Brown Leghorn hens using 30 mg. stilbestrol tablets. His results were similar to Fraps -- of 590 chicks from treated females, 57.6 per cent were females whereas in 252 chicks from controls, only 46.8 per cent were females. He noticed the same period of greatest effect and then a return to a normal ratio. Pun suggested that his and Frap's results were due either to selective elimination of the male chromosomal complement as a polar body or to sex reversal of genetic males to females by the treatment. He tried to test the latter hypothesis with 54 females of which three should have been sex reversed. These three should produce all males.

All 54 females produced both sexes, but because of the small numbers used, he still did not rule out this possibility. In 1960, Beilhary showed, at least to his own satisfaction, that sex reversal was not involved. He used for his cross, two breeds in which the sex-link gene for inhibition of dermal melanin was present in one breed but not in the other. Thus, all chicks hatched could be genetically sexed with males having white shanks and females having black shanks. The hens were treated with 15 mg. stilbestrol tablets and eggs were hatched before and during the period of maximum effect (4-5 months production). At the time of hatching, chicks were vent sexed and all cockerels and doubtfuls were discarded. No sex-ratio at hatching appeared in the data. One-hundred and ten females were produced during the 4-5 month period and shank color was not classified until maturity. He states that some of the 110 pullets did not develop marked shank color, but that they did not have the white shanks of the males. From this imperfect data he believed that sex reversal was not involved.

Lerner, et al. (Gaunt et al., 1961) reported the anti-estrogenic action of Mer-25 in blocking effectively both endogenous and exogenous estrogens in chicks.

The thyroid and its secretion level of thyroxine has an effect on egg production. Winchester and Scarborough (1953) radio-thyroid-ecrecticized some New Hampshire two-day-old female chicks with radioactive iodine. At six weeks of age, thyroid replacement therapy was started with various dosages of thyroxine. Normal or nearly normal production was achieved by the groups given 4 through 32 gamma thyroxine per unit of body size daily or 200 to 1600 per cent of normal output for growing birds. Those receiving .25 to 4.0 gamma and 32 to 64 gamma had reduced production and those receiving less than .25 gamma or more than 128 gamma did not lay at all. Other investigators feeding 0.3 per cent thiouracil (McCartney et al., 1950), or 10 grams of thyroprotein per 100 pounds of feed (Oloufa, 1953) have shown significantly reduced egg production also, thus over- and under-production of thyroxine seems to reduce egg production.

The hormones of the adrenal gland also affect egg production. An injection of 3 mg./lb. body weight of cortisone acetate stopped egg production within four days for a period of 17 days (Kudzia, 1953), whereas massive dosages (50 to 300 mg./week) resulted in adrenal and gonadal atrophy in about four weeks (Urist et al., 1960). Desoxycorticosterone acetate in excess of 10 mg./week was equal to progesterone in depressing productin (Herrick et al., 1957). Sykes (1955) using adrenaline, lowered production by 20.6 per cent with as small a dose as 0.25 mg. Dosages of 5 mg., or more, completely inhibited egg production. These adrenal hormones seem to influence the pituitary in its output of A.C.T.H. and gonadotropic hormones, particularly inhibiting the secretion of L.H.

Stress and its adrenal involvement has been suggested of accounting for the lowered reproduction rates observed in animal populations living under crowded stressed conditions. The pituitary increases its output of A.C.T.H. and stimulates the adrenal to increasing production of glucocorticoids. Due to the increased A.C.T.H. output, the pituitary shifts and reduces its output of other hormones such as the gonadotropins. Seigel (1959) tried to test this theory with White Leghorn pullets. He found over a 196-day period significantly lower egg production from the group confined to 1.33 sq. ft. per bird as compared to those with 4.00 sq. ft. per bird. The number of birds per group laying was not reduced, but the number of eggs per bird and the mean cycle length were significantly reduced.

Berry and Payne in 1961 showed a pineal body and thymus gland effect on egg production. They removed at four days of age either the pineal body, thymus gland or both. Pinealectomized birds laid significantly fewer eggs than either other group. Thymectomized birds laid at the highest rate, whereas removal of both glands resulted in production almost equal to simple thymus removal. No suggestive reasons were given.

Huston and Nalbandov (1950) showed that suspending coarse surgical thread, glass beads, paraffin balls, or pieces of cork in the lumen of the oviduct resulted in the bird ceasing to lay while, however, the follicular growth continued in the ovaries. The same operation on immature females delayed or prevented their sexual maturity. While objects were in the oviduct, injections of progesterone or L.H. caused ovulation as long as the injections continued (Huston and Nalbandov, 1953). The irritant seemed to prevent the secretion of L.H. sufficiently to cause ovulation. This would suggest the existence of a neurogenic control system between the oviduct and pituitary.

Ralph and Fraps (1960) induced premature ovulation in hens by injecting progesterone into certain regions of either the hypothalamus or the caudal neostriatum, but not by injections in other parts of the forebrain or pituitary. They (1959) placed electrolytic lesions at various sites within the hypothalamus of birds previously injected with progesterone and found that only those lesions within a ventro-median portion of the preoptic hypothalamus, or along fiber tracts passing caudal from this region regularly prevented ovulation that ordinarily would have been induced. If lesions were placed within two hours after injection, ovulation was prevented; after this time, it only prevented or delayed subsequent ovulation. van Tienhoven, Nalbandov and Norton (1954) showed that dibenamine was capable of blocking spontaneous as well as progesterone induced ovulation. It blocked most effectively 14 hours before ovulation, suggesting that the hen's L.H. is released 8 to 14 hours before ovulation. Conner and Fraps (1955) showed that phenobarbital sodium injected 22-43 hours before initial ovulation and 17-23 hours before second ovulation caused a high proportion of suppressed ovulations. In nearly every case of suppressed ovulation,

follicular atrophy was evident. The injections must have affected the mechanism involved in maintenance of the ovarian follicle, probably by way of neural pathways.

Two irradiation studies that deal directly in one case and indirectly in the other case with inhibition follow. Muller et al. (1960) irradiated in a single dose from 100 to 1000 roentgens at the rate of 43 roentgens per minute fertile eggs and one, two, and three-day-old embryos. They then selected randomly a group of female chicks that hatched from these eggs to raise to mat rity. Egg production was generally lower from these birds during the first six months of their production. Those receiving 400 to 700 roentgens exhibited delayed maturity or complete inhibition of egg production. Regression analysis showed this decrease to be 1.66 per cent for each 50 roentgens received.

Quisenberry and Atkinson (1953) gave X-ray doses of 50, 100, 200, 300 and 500 roentgens to White Leghorn cockerels of 6, 12, and 18 weeks of age. After recovery from post irradiation symptoms, they were mated to normal non-irradiated White Leghorn females. Of the embryos dying during the third week of incubation, females predominated. The per cent of third-week dead embryos was highly related to the amount of the X-ray dosage. They suggested that maybe a sex-linked lethal had been created by the irradiation.

Now to cover some of the nutritional and pharmaceutical inhibitions of egg production. There is a tremendous amount of material in the literature on protein levels and energy requirement necessary for maximum egg production which I shall not go into here other than to say that birds need adequate protein levels and energy sources for maximum production.

Feeding diets to birds which are deficient in various vitamins has a marked effect on egg production. It has been shown that a minimum requirement of vitamin A for maximum egg production for laying hens is 1200-1600 U.S.P. units per pound of diet (Hill et al., 1961), and 1200 U.S.P. units of stabilized vitamin A per pound of diet for turkeys (Stoewsand, et al., 1961). Sunde et al. (1950) showed that a ration lacking folic acid inhibited egg production completely by ten weeks in laying hens, and that 0.25 mg./kilogram of feed was a borderline level. Ringrose et al. (1961) demonstrated that niacin deficiency reduced egg production, but not to zero. Niacin at 20 to 50 mg./lb. of feed was necessary to give results equivalent to the controls. Ferguson et al. (1961) showed using Beltsville Small White turkeys (fed a basal casein-gelatin diet minus one vitamin at a time) that a riboflavin deficiency reduced production to zero in eight weeks, approached zero in the pyridoxine deficient group and suddenly dropped after 12 weeks in the pantothenic acid deficient group. In Single Comb White Leghorns, the minimum requirement of pyridoxine was approximately 2.3 mg./kilogram of diet. Below that level production was markedly reduced. Wooten

et al. (1955) have analyzed the effect of pyridoxine deficiency on the gonadotrophic content of the pituitary in rats and have found that the deficiency does not impair production of either F.S.H. or I.C.S.H. They suggest that the interference is either with the release or the utilization of these hormones. Pyridoxine may serve the same function in birds. Bird (1956) showed that the inhibitory effect of saponin in alfalfa meal can be inactivated by excess pyridoxine.

Feeding diets deficient in minerals is another way to reduce egg production. Donovan et al. (1960) fed a diet to 18 Single Comb White Leghorn hens which contained only 0.12 per cent calcium. During a five-day period, production dropped 9.6 per cent as compared with a six-week pre-experimental period. In two phosphorus studies, a diet containing between 0.18 and 0.19 per cent phosphorus caused a decrease in production (O'Rourke et al., 1953), and a diet of 0.15 per cent supported only 45.3 per cent production as compared with 69.8 per cent and 74.9 per cent production for 0.25 per cent and 0.35 per cent total phosphorus respectively (Crowley et al., 1961). Burns et al., (1952), stated that some unpublished data indicates that a potassium deficiency also decreased production. Sodium chloride below .19 per cent of the diet causes a lowering of production with sodium appearing to be the limiting factor according to Burns et al. (1952). Another study, however, has shown that a large quantity of sodium chloride, sodium sulfate and magnesium sulfate in the water will also cause significant reductions in egg production.

A large number of drugs and other substances in feed lower or stop egg production. Polybor-3, which is borax, fed at the rate of 3 and 6 lbs. per ton as a house fly larvacide reduced egg production by 10 to 15 per cent, (Sherwood, 1959, and Tower et al., 1960), and the insecticides, dipterex and diazinon depress both egg production and food consumption. (Ross et al., 1960).

Toxic-fat added to the diet reduced egg production to zero in 55 days (Dunahoo et al., 1959). When oats which had been fumigated with 64 per cent carbon tetrachloride, 29 per cent ethylene dichloride and 7 per cent ethylene dibromide at the rate of 1.5 cc. per pound were used in the diet, production was reduced to 37 percent versus 66 per cent when only 0.5 cc. level of fumigation was used (Caylor et al., 1960). Arasan, which is 50 per cent tetra-methyl-thiuram disulfide, used on corn and improved Ceresan, which is 5 per cent ethyl-mercury-phosphate, used on wheat decreased egg production. If corn made up a 1/3 of the ration, then production dropped from 60-70 per cent down to 10 per cent in 2-3 days. If wheat made up 1/2 the ration, it dropped to 25 per cent (Heuser, 1956).

Some plant products are also inhibitory. Lithospermum injected at the rate of 120 mg., 100 mg., 60 mg., and 50 mg. for five days inhibited egg laying completely in three days. Ovaries

were reduced from a control size of 59.4 gms. to 11.5 gms. This material has a definite effect on the pituitary (Zeller et al., 1958). Ground Crotalaria seed mixed with the feed at 0.2 per cent, 0.1 per cent and 0.05 per cent levels reduced production from 60 per cent to zero in 19 days, 2 per cent in six weeks and 20 per cent in six weeks in the three groups, respectively, in White Leghorn hens (Caylor et al., 1961). Feeding alfalfa saponin extracted from alfalfa at 0.4 per cent level or 0.26 per cent saponin supplied by sun-cured alfalfa meal at the 20 per cent dietary level both depressed production considerably. Recovery was rapid after discontinuation of the saponin (Heywang et al., 1959). Atropine and SKG-501 have been shown to block ovulation in the hen (Zarrow and Bastian, 1953; Gaunt et al., 1961).

Greene et al. (1961) has found that levels of reserpine which are safe for chicken breeders have adverse effects on turkey and duck breeders. Two ppm. for turkeys markedly reduces production, but not until about the third month of laying. 0.5 ppm. maintains ducks at a low level of production, but 2 ppm. and higher completely inhibits production. Feeding the antioxidant 2,5-di-tert-butyl-hydroquinone at levels of 0.25 per cent reduced production, and 0.075 per cent gave borderline results (Heywang, et al., 1958). Administration of sulfamerazine in the feed at a 0.5 per cent concentration seriously reduced production, but 0.25 per cent levels did not (Riedel, 1950). Amprolium fed to hens at levels of 0.4 per cent and 0.8 per cent reduced feed intake to 45 per cent and 17 per cent of normal and egg production to 20 per cent and zero, respectively, by the third week (Polin, 1960). Sherwood et al. (1956) showed that Nicarbazin at a level of 0.0125 per cent slightly inhibited and at 0.07 per cent completely inhibited production in about ten days. The hens returned to production in about the same length of time after being removed from the drug. Weiss et al. (1960) showed that onset of egg production can be delayed by Nicarbazin. Three histomonastatic agents have been shown to reduce production at various levels. Moore et al. (1954) states that arsanelic acid fed to turkeys continually starting at 10 or 12 weeks of age reduced production. Moreng and Bryant (1956) showed that when 4-nitrophenylarsonic acid is added to drinking water at the recommended level, one pound per 160 gallons, it reduced production from a control level of 74 per cent to 68.1 per cent. Pino and Hudson (1953) showed that feeding Enheptin to 13-week-old White Leghorn pullets for three weeks delayed sexual maturity by five weeks. Ten weeks were required to recover from the drug. Continuous treatment at 0.05 per cent level completely prevented sexual maturation. Pino (1955) inhibited egg production in laying hens with 0.10 per cent Enheptin within 7 to 10 days. Many of the birds went into a molt. After removal of the drug, production began in two weeks and was back to normal in six weeks. It has been shown that Enheptin selectively inhibits gonadotropic hormone activity, but does not affect thyrotropic activity (Pino et al., 1954).

Price and Bottorff (1954) and Price and Gingher (1956) present additional data on the effect of Enheptin upon egg production.

Before leaving the inhibitions of egg production, it should be mentioned that in poultry most diseases will stop or reduce egg production and although I do not intend to go into the subject more deeply here, the same undoubtedly applies to wild birds.

3. Additives and deficiencies in yolk formation detrimental to hatchability.

Removal of necessary substances covers a number of vitamins and a few minerals. Hens maintained on a diet furnishing them with less than 1200 U.S.P. units of vitamin A per lb. of diet laid eggs with reduced hatchability (Stoewsand et al., 1961). Lack of folic acid in the diet reduced hatchability to zero in six to ten weeks (Sunde et al., 1950, and Couch et al., 1950). The minimum required for New Hampshire pullets for normal hatchability was found to be between 26 and 34 micrograms per 100 grams of diet (Lillie et al., 1950), and for turkeys about 0.7 mg. of folic acid per kilogram of ration was required (Kratzer et al., 1956). A more acute deficiency and even lower hatchability were produced when sucrose served as the carbohydrate instead of dextrin (Sunde et al., 1950 b). Ringrose et al. (1961) reported that a niacin deficiency reduced hatchability in three weeks and caused almost complete inhibition of hatch in six weeks in chickens. Ferguson et al., (1961), reported that niacin deficiency in turkeys reduced hatchability well below 25 per cent with most of the mortality occurring during the second week of incubation. Biotin deficiencies completely reduced hatchability in both chickens and turkeys in three to four weeks (Diechert et al., 1950) with highest mortality occurring during the first week of incubation, (Ferguson et al., 1961). The minimum requirement in turkeys for pantothenic acid is about 16 mg. per kilogram of ration, lower ratios caused a drop to zero in hatchability in seven weeks (Kratzer et al., 1955) with highest mortality of embryos during the first week of incubation (Ferguson et al., 1961). A lack of vitamin B_{12} in diet has been shown by a number of workers to reduce hatchability to 14 per cent to 49 per cent (Petersen et al., 1950 and Carver et al., 1950); also Ferguson et al. (1961) showed in turkeys a similar depression and high first-week mortality. In pigeons, addition of both riboflavin and B_{12} increased hatchability by 14 per cent (Shultz et al., 1953). Lillie, Olsen and Bird (1951) showed that birds with normal high hatchability rates were less susceptible to riboflavin or B_{12} diet deficiencies than birds with low hatchability rates. Pyridoxine deficiency caused reduced hatchability in hens when the diet was below 4.3 mg. of vitamin per kilogram of diet (Fuller et al., 1961), and in turkeys it caused high second-week mortality (Ferguson et al., 1961).

Stadelman et al. (1950) showed that absence of supplementary vitamin D in turkeys also reduced hatchability. Hill et al. (1954) showed that the minimum level of riboflavin necessary in Leghorn's diet was 1.7 mg. per pound and that about 2.5 to 3 micrograms per gram of yolk in the egg was required for normal hatchability. Ferguson et al. (1961) showed in turkeys that lack of riboflavin caused completely inhibited hatchability with high first-week mortality. Maw, in 1954, found in Single Comb White Leghorns a genetic equivalent to dietary riboflavin deficiency. These birds carried a recessive gene which made them unable to transfer the vitamin from the feed to the egg. Vitamin E at 20 mg. per pound of feed was necessary for maximum hatchability in turkeys (Scott et al., 1955 and Atkinson et al.. 1961). Jensen et al. (1955) showed that feeding 2 per cent fish liver oil accentuated the vitamin E deficiency. On the basal diet he reported only 67 per cent hatchability, but addition of fish liver oil lowered this to 40 per cent.

For mineral deficiencies, O'Rourke et al. (1953) showed that a low phosphorus diet, 0.18 to 0.19 per cent reduced hatchability. Rogler et al. (1959) with an iodine deficient diet, and McCartney and Shaffner (1950) by feeding thiouracil at a level of 0.3 per cent showed similar results, but for different physiological reasons. In one case, there was no iodine for the thyroid to pick up in manufacturing thyroxine; and in the other case, the high concentration of thiouracil inhibited the thyroxine production. The end results were the same, however: the lowered metabolic rate resulted in slower growth and fewer chicks hatched.

The following drugs or materials when added to the feed all adversely affect hatchability: (Some are known to enter the yolk; the site of action of others are not known) Toxic-fats and heated and aerated cottonseed oil at a 5 per cent level (Naber et al., 1958); pure gossypol mixed with the diet at either 0.024 or 0.036 per cent levels (Heywang et al., 1950) (Bird, 1956); reserpine (in turkeys) at 2 ppm. (Greene, et al., 1961); the antioxidant 2,5-di-tert-butyl-hydroquinone at 0.25 per cent (Heywang et al., 1958); arsanilic acid fed to turkeys continually from 10 to 12 weeks of age (Moore et al., 1954); NF-180 fed to turkeys at 0.011 per cent and 0.022 per cent (Smyth, et al., 1959 and McCartney et al., 1960); and lastly, nicarbazin fed at levels of 0.005 per cent and 0.0125 per cent decreased hatchability by about 50 per cent while a level of 0.002 per cent reduced hatchability by only 20 per cent (Sherwood et al., 1956). Polin et al. (1956) found that the level in the yolk of 4,4' - dinitrocarbanilide, from feeding nicarbazin, had to be above 2 gamma per gram in order to influence hatchability.

4. Inhibition of germinal viability resulting in infertile eggs.

Germinal viability or egg fertility, is influenced by only a very small number of drugs. The following drugs affect the female's fertility: NF-180 (Smyth et al., 1959, and McCartney et al., 1960); reserpine in turkeys at 2 ppm. (Greene et al., 1961); and progesterone at 10-40 mg. per week (Herrick et al., 1955).

In oogenesis there are ample means and opportunity for one to manipulate reproductive rate. The one important limiting factor is that most of the methods mentioned require close control of the birds under treatment.

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RECENT FINDINGS IN THE INHIBITION OF AVIAN SPERMATOGENSIS

Bernard C. Wentworth

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RECENT FINDINGS IN THE INHIBITION OF AVIAN SPERMATOGENESIS

Bernard C. Wentworth

1. Introduction.

The list of agents, both physical and chemical, which affect spermatogenesis, includes changes in environment, nutrition, genetics, short wave radiation and a host of pharmacologically active substances.

The primordial germ cells in the male chick embryo begin to divide actively on or after the thirteenth day of incubation (Swift, 1916). This early germinal cell division is not a characteristic of all avian species. Kirschbaun and Ringaen (1936) found in the House Sparrow (Passer domesticus), which has been used extensively in the study of spermatogenesis, that the germinal cells begin to divide only a few weeks before the first spermatozoa mature. Like the spermatozoa of other animals, avian sperm cells normally do not show motility within the testes. The capacity to move is gradually acquired during passage through the epididymis and vasa deferentia (Anderson, 1922; Munro, 1938), a process which requires from 1 to 4 days in the chicken, depending on mating activity.

The spermatozoa of passerine birds are distinguished by one outstanding feature, namely, their predominantly spiral configuration.

2. Environment

The observation by Rowan (1925), that the testes of the Junco (Junco hyemalis) can be stimulated during the winter by increasing the day length with artificial light has stimulated extensive investigation. The seasonal nature of reproductive activity in birds is due to the extrinsic factor of light affecting the gonads which acts through the mediation of the anterior pituitary gland (Benoit, 1935).

In practically all wild birds, reproductive activity is a seasonal phenomenon. Breeding generally occurs during the spring and early summer. The gonads then regress to a quiescent state and remain inactive until the approach of the breeding season of the following year. Domestic birds do not conform strictly to this cyclic pattern, although as Parker and McSpadden (1943) demonstrated, domestic chickens produce the greatest density and volume of semen in April at a mean temperature of 52°F. They also found that the lowest fertility was obtained in September at a mean temperature of 68°F. Gamal et al. (1959) found the lowest semen quality in December and January.

A diurnal variation in spermatogenic activity has been observed in the domestic fowl by McCartney (1942) and Riley (1940). McCartney found the peak activity at midnight and Riley found the peak activity at 3 a.m.

The testes of wild birds are extremely sensitive to variations in the diurnal light regimen which must exceed a certain minimum. In the Starling (Sturnus vulgaris) 12.5 hours of light is required (Burger, 1940). Photoperiodism in birds does not in general permit a distinction between the effects of a long light period and that of a short, dark period (Kirkpatrick and Leopold, 1952). According to Fenner and Engels (1952) a night interruption will also induce sexual maturation in juncos and White-throated Sparrows (Sonotrichia albicollis). Carson et al. (1958) found that red and gold illumination treatment significantly suppressed subsequent sperm production of cocks as compared with the incandescent lighting used on the controls.

Evidence for the cyclic pattern of gonad activity seems to be associated strictly with the lengthening day if one does not consider the exceptions. Chapin (1954) reported evidence compiled over a period of eleven years that showed the Sooty Terns (Sterna fuscata) on Ascension Island assemble to begin nesting at an average interval of 9.6 months. In other words, they nest 5 times every 4 years. The reason for this unusual cycle of reproduction does not seem to be associated with any weather or any light intensity changes on the island. Another exception is the male parakeet (Melopsittacus undulatus) for Vaugien (1953) found fledgling males isolated in complete darkness show a greater increase in gonad weight than birds kept under natural light.

Threadgold (1960) found that in the House Sparrow, 7 foot-candles of light for one hour daily was more effective in stimulating spermatogenesis than the same amount of light for 7 hours. In both cases, a full testicular cycle was induced, but spermatic activity was maintained for the longest period in those birds receiving the single hour of light. Using growing chicks Moreng et al. (1956a) found a statistically significant increase in comb and testes weights in the group grown in a dark environment as compared to those grown in intense light (Table I).

Table I**

Organ Weight of five-week-old S.C. White Leghorn Cockerels

	Comb wt. (gms.)	Adrenal wt. (mgm.)	Testes wt. (mgm.)	Thyroid wt. (0.1%Thiouracil) (mgm.)
Light 100W (Fluorescent)	1.75	59.0	140.0	375.0
Dark 7½W	2.83	63.0	173.0**	432.0

** Moreng et al. 1956.

A greater pituitary activity in general was indicated in this study since there was also an increase in thyroid and adrenal weight in birds grown in the dark environment. There is considerable evidence that birds become refractory to light by an over-stimulation of the pituitary which becomes exhausted of gonadotropic hormones (Miller, 1948, 1949).

3. Nutrition

Another factor which may be considered as part of the bird's environment is nutrition. Parker and McSpadden (1943) found that a reduction in food consumption curtails the production of spermatozoa by Rhode Island Red chickens. A deficiency in essential feed components may lead to low fertility or sterility. A deficiency of B-complex vitamins leads to testicular degeneration in pigeons. Thiamine appears to be more important for testis maturation than riboflavin. When chickens are fed for a prolonged period on a diet deficient in vitamin E, involutionary changes may occur in the testes, with degeneration of cellular elements in the reverse order from which they are formed (Harris et al., 1954). Birds as compared to mammals are very resistant to vitamin E deficiency. The part of the germinal cell affectedby vitamin E deficiency appears to be the fat or fatty acid bodies within the germ cells (Adamstone and Card, 1934). Vitamin E appears to have direct effect on the pituitary, since Herrick et al. (1952) found considerable evidence of less gonadotropin and fewer basophilic cells.

4. Genetics

Since the spermatozoan itself is the means whereby the traits of the sire are conferred upon the sons and daughters, it is not surprising that genetic factors may alter the normal spermatogenesis. Jones and Lamoreux (1942) studying high and low fecundity strains of White Leghorn males by histological examination of the testes, found that there were more metaphase plates and spermatozoa present in the seminiferous tubules of the testes in males of the high fecundity strain. It appears from this investigation that the rate of sperm cell formation is as much under the influence of heredity as is the rate of egg formation. Goodwin et al. (1955) suggested that individual differences in pituitary output of gonadotropin is under the control of genes.

The phenomenon of parthenogenesis has been extensively studied by Olsen (1960a, 1960b) in turkeys. He found that all parthenogenetic embryos were males and of the few that hatched and reached maturity, three produced spermatozoa and one sired offspring. This one male was used to inseminate 7 virgin hens which produced 189 eggs of which 49.7 per cent were fertile and 85.1 per cent of the fertile eggs hatched. The poults were

equally divided between males and females. Fertile eggs were found 50 days following a single insemination, which indicated a normal duration of fertility. Olsen recently reported (1961) that the frequency of parthenogenesis in turkey eggs could be significantly increased by Rous sarcoma virus.

Another alteration of the normal genetic endowment is the phenomenon of sex reversal. Newcomer et al. (1960) conducted an investigation of a genetic female which had laid eggs while on the farm, but later developed the secondary sexual characteristics of a male. They found one gonad on the left filled with tubules active in spermatogenesis. The morphology and size of the spermatozoa, however, were so unnatural as to indicate little, if any, functional possibilities. They suggested that this unnatural morphology and size indicated polyploidy.

A final alteration in spermatogenesis as related to genetics is that found in hybrids. Many observers are in agreement that spermatogensis in hybrid birds may be fairly normal up to the first reduction division. The second reduction division frequently does not occur in hybrid birds such as domestic dove and magpie pigeon cross; instead, the secondary spermatocytes become converted into giant spermatids which in turn are transformed into spermatozoa of approximately twice normal size. Sterile hybrids of the muscovy duck and mallard duck occasionally undergo normal meiosis which proceeds through the first maturation division. However, the resulting secondary spermatozoa degenerate (Crew and Koller, 1936; cited by Romanoff, 1960). Asmundson and Lorenz (1957) reported that over 30 each of turkey-pheasant hybrids and chicken-pheasant hybrids raised were all sterile. In reports of avian hybrids, males predominate.

5. Hormones.

The activity of the bird's gonads is controlled by the secretion of the pituitary gland. This gland, stimulated by neural reception of light results in secretion of gonadotropin, which in turn acts upon the testes. The importance of the pituitary can be illustrated by its removal, which causes testicular atrophy in birds (Hill et al., 1934). The administration of either whole pituitary extracts or of purified F.S.H. causes enlargement of the testes. Wild birds out of breeding season are unresponsive to administration of gonadotropic hormone.

Androgens depress the pituitary and the resulting lack of gonadotropic hormone causes the involution of spermatocytes, but not of spermatogonia (Kumaran and Turner, 1949). Moreng et al. (1956b) found that injection of estrogen into the male, reduced testis weight and inhibited production of spermatocytes, presumably by depressing secretion of pituitary gonadotropin (Kumaran and Turner, 1949, Moreng et al., 1956b). van Teinhoven (1957) found that testis weights were significantly decreased

in males from eggs dipped in 5 mg. D.E.S./100 ml. of oil. Also, there was a delay in spermatogenesis as compared to controls (Table II).

Table II**

Effect of Hormone Treatment of the Eggs on Reproductive Organs of Genetic Males. (Males 6 months of age)

Treatment	Body wt. (gm.)	Gonad wt. (gm.)
Control	3,712	17.07
Oil emulsion	3,503	21.16
D.E.S. 5mg./100ml. oil emulsion	3,545	9.19*

*significant at the 0.01 level
**van Teinhoven (1957)

Herrick and Adams (1956) found that progesterone and/or diethylstilbestrol depressed testes weight (Table III).

Table III**

Testes Weight in Grams and Spermatogensis of S.C. White Leghorn Males Treated with D.E.S. and/or progesterone

Control*		Progesterone		D.E.S.		D. & P.	
wt.	sperm	wt.	sperm	wt.	sperm	wt.	sperm
20.8	+	10.6	R	1.4	-	0.7	-

* 21 birds used in each treatment
** Herrick and Adams (1956)
+ normal R reduced - lacking

The administration of progesterone or prolactin is antagonistic to gametogenesis, and also inhibits gonadotropic hormone secretion (Bates et al. (1937). Fox (1955) also noted that progesterone reduced comb index and testis weight of S.C. White Leghorns when progesterone was injected weekly. In the absence of the thyroid gland, Brown Leghorn testis development was subnormal and spermatogenesis did not proceed beyond the formation of secondary spermatocytes (Blivaiss, 1947). Although Mellen and Wentworth (1962) noted some mating activity of radiothyroidectomized males, they did not present any data pertaining to spermatogenesis. Adrenaline appears to have an unfavorable influence on the gonads. Its injection has been found to affect the production of spermatozoa very adversely in domestic cocks (Wheeler et al., 1942) and in House Sparrows (Perry, 1941). Cortisone induced precocious spermatogenesis in cockerels and Pekin ducks (Leroy, 1952; cited by Romanoff, 1960).

The use of anti-gonadotropic serum produced by immunization techniques will cause inhibition of spermatogenesis and subsequent testis degeneration in mammals. A similar phenomenon may be exhibited in birds although there have been no encouraging reports (Tyler, 1961).

6. Drugs.

There is a host of pharmocologically active substances that affect spermatogenesis of birds. Hudson and Pino (1952) found that Enheptin (2-amino, 5-nitrothiazole) used at a 0.1 per cent level in feed reduced testis size and spermatogenic activity (Table IV). They reported that 41.0 mg./kg. body weight caused complete suppression of sexual development in White Leghorn cockerels. When the Enheptin treatment was discontinued,

Table IV*

Comb and Testes Changes in Control and Enheptin Fed White Leghorn Cockerels. (Average of 8 birds)

	Comb size start (cm)	Comb size finish (cm)	Gain or loss (cm)	Testes wt. (gm)
Control	50.6	80.7	+ 30.1	14.72
Enheptin	59.0	30.9	- 28.1	0.38

* Hudson and Pino, (1952)

approximately 10 weeks ~~was~~ were required for recovery. These workers postulated that the action of the drug may be a pituitary involvement. Levis and Smyth (1957) reported that 0.1 per cent Enheptin fed to turkeys from 8 weeks of age, delayed spermatogenesis and age of sexual maturity.

Zeller et al. (1958) found that lithospermum injection stopped egg production and reduced the size of comb, ovary and oviduct. Using lithospermum treatment in mammals, Drasher (1949) and Noble et al. (1954) found that the gonadotropin titer of the pituitary was reduced. They suggested that there was an inhibition of L.H. activity.

Parizels (1960) in an extensive study with mammals found that salts of heavy metals caused testicular necrosis. Cadmium salt (0.04 moles) resulted in increase of testis size in mammals a few hours after injection. This enlargement was followed by complete testicular necrosis. The animals took on the appearance of castrates. He concluded the effect was not through the pituitary.

Retarded sexual maturity of turkeys following treatment with 0.011 per cent furazolidone was observed by Redman and Smyth (1957). Weiss et al. (1960) found that 0.04 per cent Nicarbazine retarded sexual maturity of White Leghorns. Another pharmacological treatment is the use of anti-cock-rabbit serum reported by Belenjkii (1940); cited by Romanoff,(1960) as resulting in the absence of spermatogenesis and an increased amount of interstitial tissue in the testes. Triethylenemelamine (TEM) at 0.2 mg./kg. daily for five days has a radiomimetic effect on spermatogonia in the male rat (Steinberger et al., 1959) and is currently being investigated at several laboratories as a bird sterilant. More promising perhaps than TEM are methylethanesulphonate and methylmethanesulphonate which inhibit spermatids and spermatozoa (Jackson et al., 1961) thus acting quickly after administration.

7. Short wave radiation.

Yamamoto (1959) noted that irradiated fatty acid from liver given to rabbits caused the degeneration of germinal cells with the formation of giant cells or multinucleated cells and mitotic abnormalities.

The male germ cells of the chicken appear not to be affected when the embryo is subjected, before incubation, to x-ray doses ranging from 40r. to 480r. or when doses of 100r. to 400r. are given during incubation. However, in make chicks less than 3 weeks old, irradiated with dosages in excess of 200r., the division of spermatogonia was arrested, the nuclei of these cells became pycnotic and the cytoplasma was pale (Essenberg and Zikmund, 1938). In adult cocks nearly 1300r. was required for initial injury, revealed by pycnosis of the nuclei in primary and seoondary spermatocytes and by a decrease in the number of spermatids (Essenberg and Kanasch, 1940). Sturkie et al. (1949) found when x-rays are administered before sexual maturity, a dose of 2100r. is the minimum that prevents subsequent spermatogenesis in most individuals. They observed that within 14 days after administration of a sterilizing dose, degenerate spermatozoa were seen in the lumina of many tubules and within 25 days only one layer of cells remained lining the tubules. Regeneration of spermatogenic tissue does not occur. It is of interest to note that irradiation of cock semen in vitro with a dose up to 10,000r. has been shown by Kostin, (1960), to have no detectable effect on the motility, respiration and anaerobic glycolysis, although the fertilizing capacity is reduced (Mann, 1954).

Ershoff and Brat, (1960), experimenting with rats, found that x-irradiation and gamma irradiation from a cesium 137 source were comparable in effects upon the development and morphology of the reproductive system, both irradiations producing a significant degree of gonadal injury.

8. Summary

Spermatogenesis has been shown to be inhibited by a variety of ways. Reduced quanta of light, vitamin E deficiency, altered hormonal balance, spontaneous hybridization and sex-reversal all appear to influence varying degrees of spermatogenic inhibition.

Inhibition of spermatogenesis has been induced by shortwave irradiation, drug treatment and as an immune response.

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INHIBITION OF AVIAN EMBRYO SUSTENTATION.

Raymond P. Coppinger

1. External stimuli regulating nesting cycle.

2. Internal regulation of nesting cycle.

3. Literature cited.

INHIBITION OF AVIAN EMBRYO SUSTENTATION

Raymond P. Coppinger

Inhibition of avian embryo sustentation does not have as strict a demarcation in the annual cycle of the avian species as do the other papers presented in this symposium but has an overlap into other areas of the behavior and physiology of birds. The ultimate success or failure of progeny does not occur exclusively during that period spanning egg deposition and chick emergence from the egg. Therefore, in order to handle the subject comprehensively, I will consider that period in the annual cycle which begins at the time the potential parent is ready to take up breeding territory, and ends roughly at the time the "embryo" is ready to leave the territory.

1. External stimuli regulating nesting cycle.

Although the reproductive activities are triggered in the spring, there are many environmental factors that have a regulatory effect on the success or failure of the nests. It has been confirmed by Wolfson (1959) that the lengthening of the light period in the spring initiates the reproductive activities and causes and regulates the gametogenesis of many species of birds. Marshall (1954) recognizes this light factor but qualifies its affect upon the complexity of events in the sexual life of the bird. Environmental factors--potential predators, weather, food, and the availability of nesting sites--are a few of the factors which regulate, augment, or inhibit the nesting activities. Marshall (1954) showed that on Jan Mayan Island, most species never successfully nested because of disturbance by the fox population. However, even these non-breeding birds went through a gametogenic cycle.

Kluijver (1961) found that a cold spell set back the egg production of the Black-capped Chickadee (Parus atricapillus) for a week in Massachusetts. Some of these birds had already begun to nest, but they ceased laying activity during the cold weather; others were suspected to be ready, but postponed laying until after the cold period. Kluijver noticed the same thing in previous studies,Kluijver (1951), of the Great Tit. Paynter (1954) reported that a severe storm caused Tree Swallows (Iridoprocne bicolor) to cease laying for a brief period or even to abandon nests after an egg had been layed. This does not necessarily mean that the swallows did not renest elsewhere.

In some bird species, ovulation may not take place until the traditional food supply becomes abundant, even though the birds may be physiologically ready. Marshall (1954) pointed out considerable evidence that most bird species breed at the height of the protein food supply, requisite for the rapid growth characteristic of passerine birds.

Social interaction and external stimuli play a part in the nesting cycle, and have a direct effect on physiology and pertinent behavioral conditioning. Emlen and Lorenz (1942) implanted wild California Quail (Lophortyx californicus) with sex hormone pellets and found that the untreated birds which ranged with the treated birds showed similar accelerated sexual development through social interaction. Darling (1938) and Paynter (1949) both suggest that the clutch size in gulls' nests increased with an increase in the total number of birds in the colony. Lehrman et al. (1961) working with Ring Dove (Streptopelia resoria) introduced a mate and nests into cages with sexually inactive birds. He found that the addition of either a mate or a nest produced sexual and/or incubation behavior in both sexes. Oviducts were found to have increased in weight and size, as compared with those of females which were kept alone throughout the same period. Burger (1953) showed testis growth in Starlings (Sturnus vulgaris) was greater in males caged with females than in those kept with other males. F.H.A. Marshall, as reported by Marshall(1954) suggested that these effects of social stimulation are mediated through the hypophysis and consequent response of the gonads. Bulough (1945) and Collias (1950) review the dependence of sexual behaviorisms upon androgens and estrogens of the gonads. Courtship singing and posturing by the male, shown by Collias (1950) to be caused by androgen, stimulates the receptivity of the female, and as Lehrman has noted, the change in the female's behavior restimulates the male to other phases of the nesting cycle. Males come into full sexual or breeding condition first, in most species, and continuance of the nesting cycle is then dependent upon the responsiveness of the female. Marshall (1954) pointed out that the female's responsiveness may be influenced by other external factors. Marshall and Disney (1957) showed that the African Red-billed Weaver (Quelec Quelea) nested only after rain had produced green grass, which is the traditional nesting material of this bird. Hinde and Warren(1959) found that by depriving nesting materials and space, they could retard egg laying in canaries. Lehrman's (1961) inducement of ovulation and the appropriate incubation behavior in the Ring Dove simply by means of addition of eggs and nest is pertinent.

2. Internal regulation of nesting cycle.

One of the physological reactions which is noted early in the nesting cycle is the build up of calcium in the blood and bone which is necessary for egg shell production. Clavert and Benoit (1942), as reported by Simkiss (1961) showed that the injection of estrogens (.25 mg. per day) into 3-month-old male pigeons produced a 60 per cent increase in skeletal calcium. Common, Rutledge, and Hale (1948) showed that both estrogens and androgens were needed to cause calcium increase in females. Eisner (1961) indicates these two hormones are present in both male and female as gonadal development takes place. Simkiss

(1961) states that this increase in skeletal weight in pigeons is due to the partial ossification of the marrow portions of the bone, called the medulary bone. This medulary bone has been reported in many species of birds. In the pigeons, medulary bone calcium was completely reabsorbed when injection of androgen and estrogen ceased.

Estrogen produces another change in the bird, namely, the appearance of the brood patch, which is common to many species of birds. The brood patch is a defeatherized ventral apterium with increased vascularization of dermal tissue in that region. Bailey (1952) injected finches with estrogen, producing a complete brood patch. There was no effect resulting from the injection of prolactin, a pituitary hormone. Bailey then hypophysectionalized a number of birds and treated some with estrogen and some with prolactin. The estrogen-treated birds attained increased vascularization of the dermis in the ventral apterium, but no defeatherization. Upon treating these birds with prolactin, defeatherization occurred, and a complete brood patch was formed. Bailey then concluded that estrogen elicits the pituitary to secrete prolactin, which completes the formation of the brood patch. Eisner (1961) pointed out that Bailey did not check the effect of progesterone, which might be involved in this process. It is believed that prolactin influences the secretion of progesterone, as shown by Meites and Shelesnyak (1957). Lehrman (1955) showed that the primary effect of prolactin is engorgement of the crop with pigeon-milk and suppression of sexual behavior in the Ring Dove. Eisner (1961) points out that repression of sexual behavior during incubation is due to the anti-gonad effect of prolactin on androgen production. Bailey,(1952), working with California Gulls (Larus californicus) showed that prolactin secretion in the male begins at the time the testes begin to regress. Hormonal factors leading to the production of the brood patch also affect behavior of the bird. The brood patch may not be a necessity for incubation, although in most species the presence of brood patches appear to correspond with participation in incubation. Skutch (1957), however, showed that this correspondence does not necessarily hold in passerines. Bailey (1952), for example, induced brood patches in both male and female passerines, where normally the male does not have it. Kluijver (1961), however, observed that the male Chickadee normally developed a brood patch, although he does not incubate. Verner (1961) working with the Red-footed Booby (Sula sula) found that neither parent developed a brood patch, although both incubated. The birds used either their feet to warm the eggs, or tucked the eggs into ruffled feathers. Weston (1947) found that the male Black-headed Grosbeak (Pheucticus melanocephalus) incubated 40 per cent of the time during the day, but Bailey found no incubation patch in that species. Petersen (1955) working with Bank Swallows (Riparia riparia) of which both parents incubate equally day and night found that males did not have an incubation patch. Lehrman (1955) suggested that

birds with highly developed brood patches find relief from "peripheral irritation" by sitting on eggs.

Baily (1952) working with California Gulls and several passerines, and Saeke and Tambe (1954), working with pigeons, showed a relatively high prolactin content of the pituitary as long as nest building continued but low content during laying. Daily intermuscular injections of fifteen I.U. of prolactin induced full broodiness when, but only when, birds were molting. Lehrman (1961) suggested that progesterone is directly concerned with incubation behavior but once the behavior is induced, prolactin secretion begins. Blakely et al. (1951) using doses of 200 mg. of diethylstilbesterol were effective in breaking up broodiness in turkeys. Eisner (1961) noted that dosage levels are critical, for small doses of progesterone induce ovulation, while larger doses inhibit it. Kosin (1948) injected 10 mg. testosterone propionate and failed to break up already broody turkeys although in laying birds it seemed to prevent broodiness. Bailey (1952) reported that androgens had no effect on the brood patch. Laying, incubation, and molt usually occur in sequence but are not necessarily action-and-reaction processes. Some of these processes occur simultaneously, such as incubation and molt in some species. Broody birds renest if the nest is destroyed or they can adopt a new mate if pair-bond is broken; in both cases necessitating a turning-back of the physiological clock. Once incubation has begun, it must continue until the eggs are hatched. It is obvious that eggs are the stimulation that is needed for if the eggs are removed, incubation behavior ceases. Weidmann (1956) induced incubation in gulls by placing dummy eggs in the nest just before laying and thereby prevented laying. Tinbergen (1954) also working on gulls removed the eggs a short distance from the nest, and showed that both eggs and nest stimulated incubation behavior for the bird incubated first the eggs, and then the nest. The gulls did not, however, attend to only the nest itself when no eggs were on the territory. Tinbergen also showed that one bird which lost its mate abandoned the eggs which indicates that perhaps a mate is needed for the completion of the cycle in gulls. Patel (1936) working with pigeons came to the same conclusion. Patel experimentally separated a male from its mate but both sexes were kept visible to each other. Both progressed normally, developing the necessary "milk" at hatching time. When the mates were removed from visual contact, however, incubation behavior was lost in the male.

Sturkie (1954) reviewed how light-stimulation and other mechanisms affect the action of the pituitary. For purposes of this paper, I will discuss the actual visual stimulation which causes these external effects. We have seen that environmental stimulation helps to regulate the commencement of the nesting cycle. These mechanisms through natural selection assure the maximum success from an environmental point of view for the

raising of the brood. Paynter (1949) and Lack (1954) showed that late and second broods in gulls and swifts were less successful either through asynchronization in the colonies, dwindling food supply, or lack of strong attentiveness on the part of the parent.

Clutch size may or may not be regulated by visual or tactile response. To quote David Lack (1954), "The restriction of clutch size is due not to a negative, the inability to produce more eggs, but to a positive act, the cessation of laying, which often involves the reabsorption of further eggs already formed internally, but not yet laid."

Eisner (1961) concluded that the most obvious physiological control of termination of laying is a rise in the level of prolactin or progesterone. In different species, there are three categories of clutch size. The first category is the fixed clutch size: gulls, petrels, terns and related species, lay a specific number of eggs, the number is not significantly affected by environmental factors (Lack, 1954). Tinbergen (1960) showed that the removal or addition of eggs to Herring Gulls' nests did not induce the gulls to lay more than or less than three eggs. Either a determined number of follicles begin to mature, or there is an accumulation of progesterone, with a given number of ovulations, or an inhibitory prolactin level is reached during this period, thus laying is inhibited without dependence on external stimuli. Weidmann (1956) showed that more than three follicles began maturation in gulls. If the gulls incubated the first clutch, other follicles did not develop. Although the clutch is three in theory, there are many two-egg nests. Darling (1938) relates this to density dependent variations. The more gulls there are nesting, the greater the stimulation per pair. Synchronization is better in large colonies than in small ones. Social stimulation speeds up the process and shortens the total nesting period. Palmer (1941) found this to be true in Common Terns (Sterna hirundo) also but cautions that the advantages gained from increased stinulation may be offset by overcrowded colonies that there is probably an optimum colony size.

The second category of clutch size determination is a governing of the number of eggs layed by an attempt of the laying bird to seemingly reach a minimal quota. Lack (1954) reports that a Flicker (Colapter aurcitus) was induced to lay 71 eggs; she laid an egg a day for 71 days, to replace the egg a day which was removed from her nest. Wetherbee and Wetherbee (1961), however, could not corroborate this often cited experiment. The domestic hen is an excellent example of this category for the hens lay for months when the eggs are removed from the nest.

A third category of clutch size determination is dependent upon environment. In raptorial birds and storks, the clutch size goes up in correlation with the food supply (Lack,

1954). In a paper by Lack and Arn (1947), as reported by Lack, 1954, on the Alpine Swift (Apus melba) a correlation was drawn between clutch size and survival rate.

In summary: both hereditary factors and environmental factors influence the clutch size.

I have spent much time on these events since the survival of the egg in the nest is almost completely dependent on the success of the parent in displaying the proper behavioral responses to various situations, and in coping with her environment. Manipulation of these responses can result in thwarting of the sustentation of the embryo. We find in the field that although adverse weather conditions, wind, rain, temperature and humidity may play a part in the success of the brood that the parent compensates for most of these impingements. Moring and Bryant (1954) submitted domestic chicken eggs at various incubation stages to -10°F. temperatures for various lengths of time. Prior to the 17th day, a temperature of -10°F. for 85 minutes showed very little effect on hatchability. It is hard to imagine this situation occurring in the field. Romijn and Lokhorst (1955) showed that egg temperature is slightly cooler than the environment up to the 10th day of incubation, and slightly warmer than environmental temperature from about the 12th day on. MacMullan and Eberhart (1953) working with wild pheasants showed that as the temperature is lowered, the endurance of the embryo became shorter; the older the embryo, the more vulnerable to exposure it became. These authors showed that tolerance to cold in pheasants was very great and that embryo mortality in the wild attributable to cold is precluded by the attentiveness of the parent. Severe cold and rain at the time of hatching, however, is definitely a decimating agent.

Kessler (1960) and Farner (1958) showed that there is greater attentive efficiency on the part of the parent as incubation progresses. Kessler reported that pheasant egg temperatures under the incubating parent did not reach a high point until the 12th, 13th, or 14th day. Farner working with the Yellow-eyed Penguin (Magadyptes antipodes) observed that the temperature of the egg increased from the 2nd to the 15th day. This rise in temperature coincided with the vascularization of the ventral apterium. He suggests that this relatively long warm-up period may play a part in the relatively long incubation period of this species. Farner's review of the literature revealed that this is common in other species. It was shown by Holstein (1950), as reported by Farner (1958), that the peak temperature in Goshawks (Accipter gentilis) did not come until the 22nd day. The Sparrow Hawk (Falco sparverius) has a similar warming-up period. Bergmann (1946), also reported by Farner (1958) working with turnstones, showed that the temperature increased from 30°C. to a maximum of 38-40°C. by the 18th day. All these birds have a relatively long incubation period.

The greatest loss of eggs and nestlings in a wild population is through predation. Tinbergen (1960) showed that as predation increased, gulls spread their colony over larger areas, which because of the criptic coloration of the eggs and chicks, and the harrassment of the adults, made it more difficult for the predator to locate the nests.

Physical discomforts are not so apt to be lethal to nestlings as might be supposed. Barth (1951) found that gulls could withstand temperature ranges of 16°C. to 44°C. If the temperature was held between 41°C. and 43°C., four-day-old birds died in 38 minutes. An eight-day-old bird reached a temperature of 44.2°C. in 10 minutes, but did not die.

Weather plays a role in the survival of the young but survival can usually be related to food. Lack and Arn (1947) and Lack (1951), as reported by Lack (1954), found that the Alpine Swift had greater success in dry years than in wet, foggy years because in wet years the insect food was lower, and much more difficult to obtain.

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